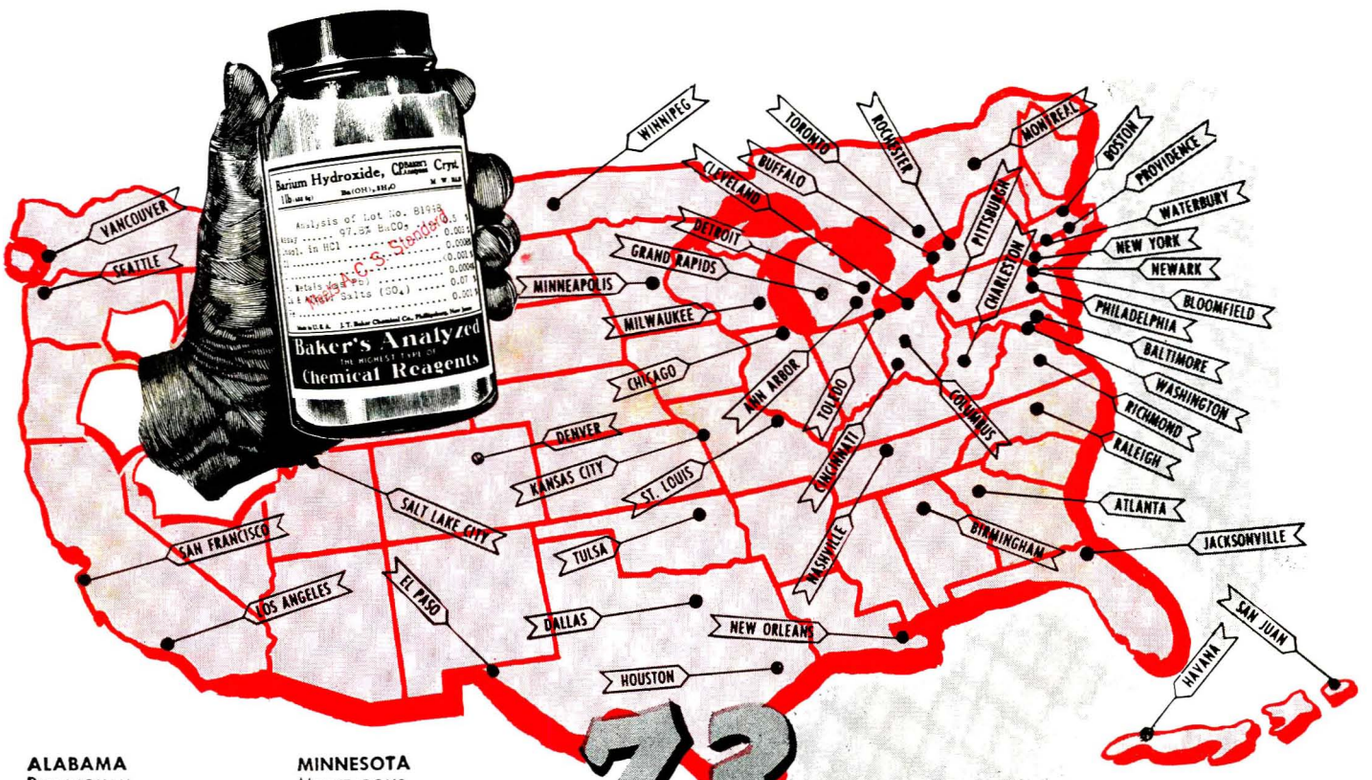
ANALYTICAL CHEMISTRY

MARCH 1951



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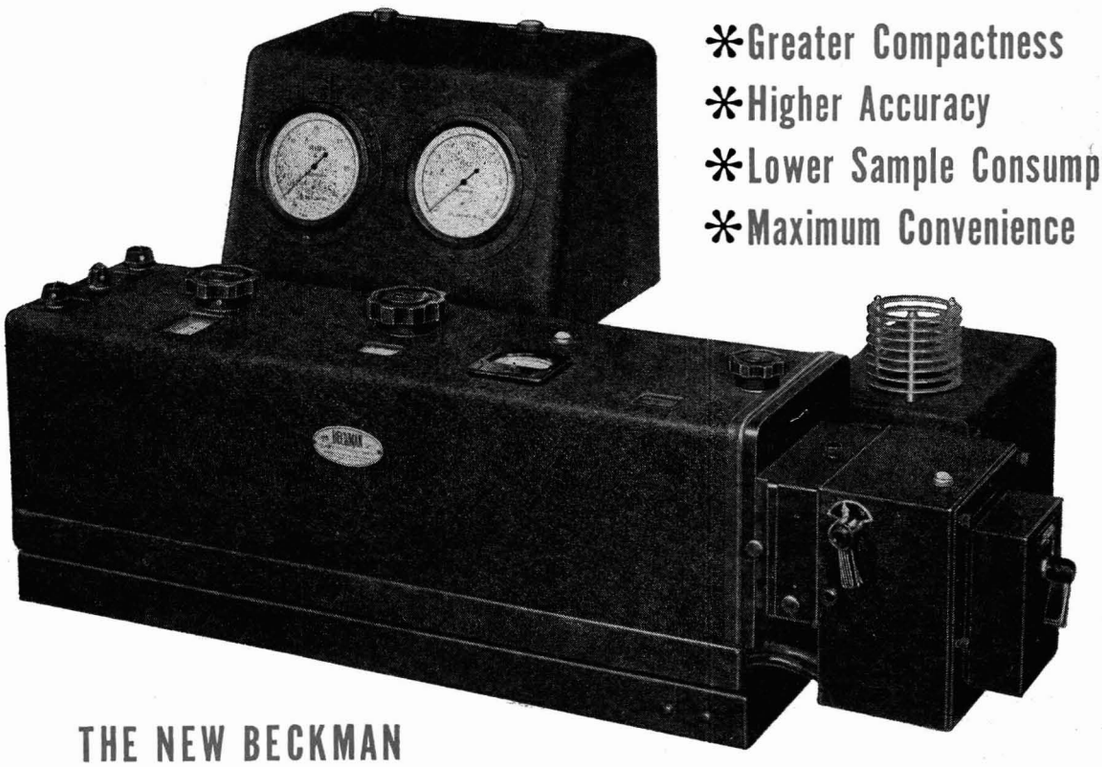

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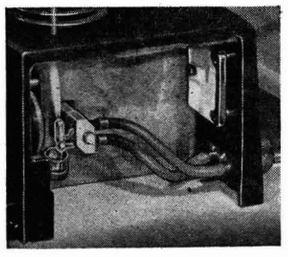

The atomizer-burner of this new instrument is much smaller, simpler and more trouble-free than previous designs. Made of Pyrex with a straight, large-diameter, noble-metal atomizer tube discharging directly into the flame, it will spray even cloudy or highly concentrated solutions indefinitely without clogging or "drifts".

Other important features of this new Beckman development are outlined at right. Best of all, this new instrument is available at a new low price for equipment of this quality. Your nearest authorized Beckman Instrument dealer will gladly supply full details—or write direct!

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- The hot flame, coupled with the high resolution of the "DU" Spectrophotometer, permits unusually narrow band widths to be used—less than 10 millimicrons for most determinations. Accuracies of 0.5% or better are obtainable.
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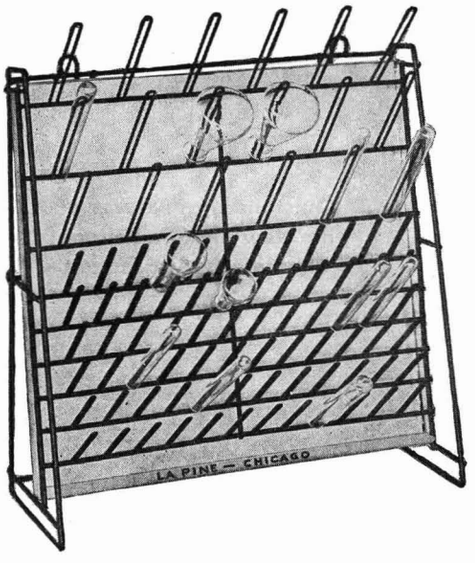

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- Economical and sturdy—will last indefinitely.
- Neoprene coated, resistant to many chemicals and oils.
- No warping—no absorbing of liquids.
- All drainings caught in rust-resistant pan. No mess.

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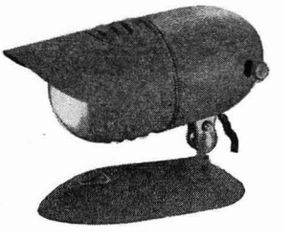

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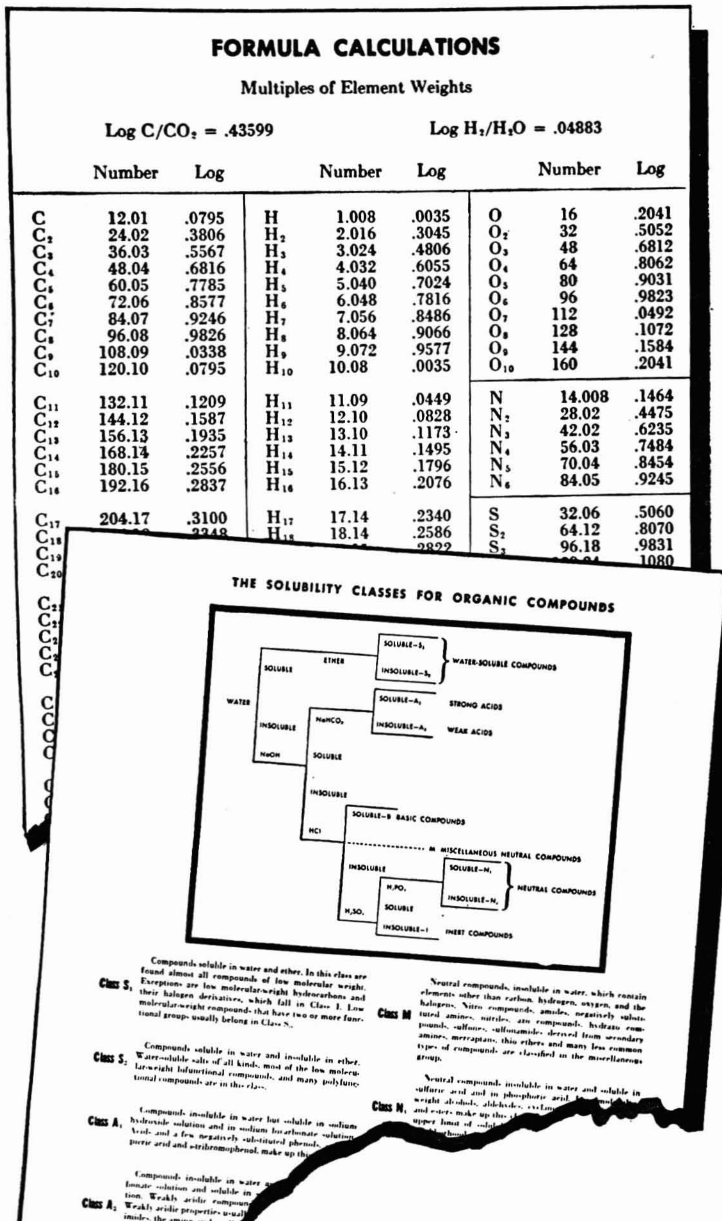

FORMULA CALCULATIONS

Multiples of Element Weights

Log C/CO_2 = .43599 Log H_2/H_2O = .04883

	Number	Log		Number	Log		Number	Log
C	12.01	.0795	H	1.008	.0035	O	16	.2041
C_2	24.02	.3806	H_2	2.016	.3045	O_2	32	.5052
C_3	36.03	.5567	H_3	3.024	.4806	O_3	48	.6812
C_4	48.04	.6816	H_4	4.032	.6055	O_4	64	.8062
C_5	60.05	.7785	H_5	5.040	.7024	O_5	80	.9031
C_6	72.06	.8577	H_6	6.048	.7816	O_6	96	.9823
C_7	84.07	.9246	H_7	7.056	.8486	O_7	112	.0492
C_8	96.08	.9826	H_8	8.064	.9066	O_8	128	.1072
C_9	108.09	.0338	H_9	9.072	.9577	O_9	144	.1584
C_{10}	120.10	.0795	H_{10}	10.08	.0035	O_{10}	160	.2041
C_{11}	132.11	.1209	H_{11}	11.09	.0449	N	14.008	.1464
C_{12}	144.12	.1587	H_{12}	12.10	.0828	N_2	28.02	.4475
C_{13}	156.13	.1935	H_{13}	13.10	.1173	N_3	42.02	.6235
C_{14}	168.14	.2257	H_{14}	14.11	.1495	N_4	56.03	.7484
C_{15}	180.15	.2556	H_{15}	15.12	.1796	N_5	70.04	.8454
C_{16}	192.16	.2837	H_{16}	16.13	.2076	N_6	84.05	.9245
C_{17}	204.17	.3100	H_{17}	17.14	.2340	S	32.06	.5060
C_{18}			H_{18}	18.14	.2586	S_2	64.12	.8070
C_{19}						S_3	96.18	.9831

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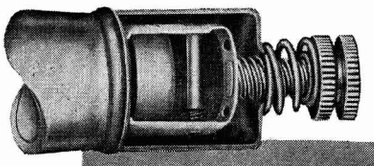
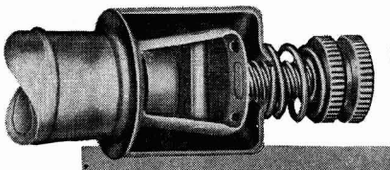
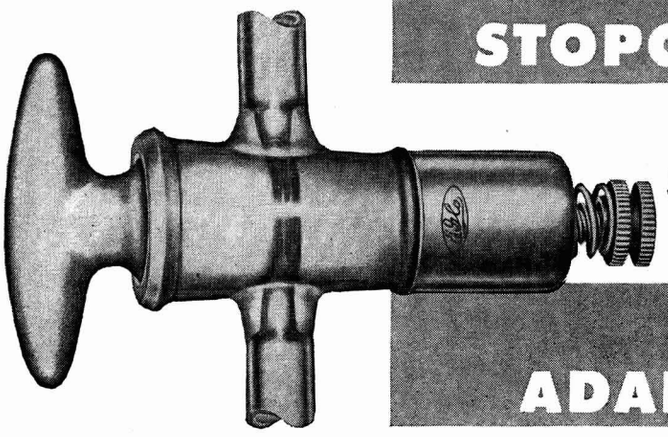
This single size  adapter

UNIVERSAL

converts ANY  glass stopcock

STOPCOCK



to pressure-tight stopcock

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detailed description

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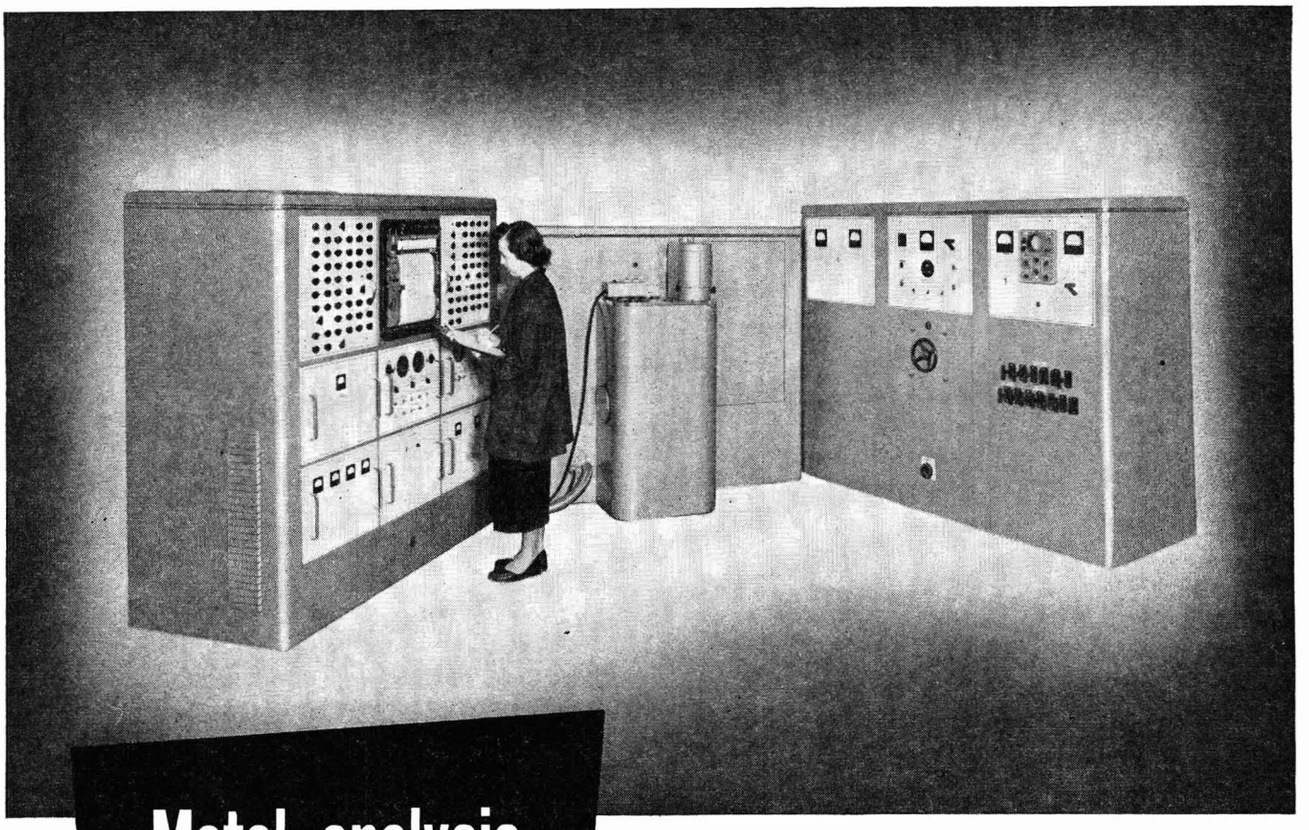

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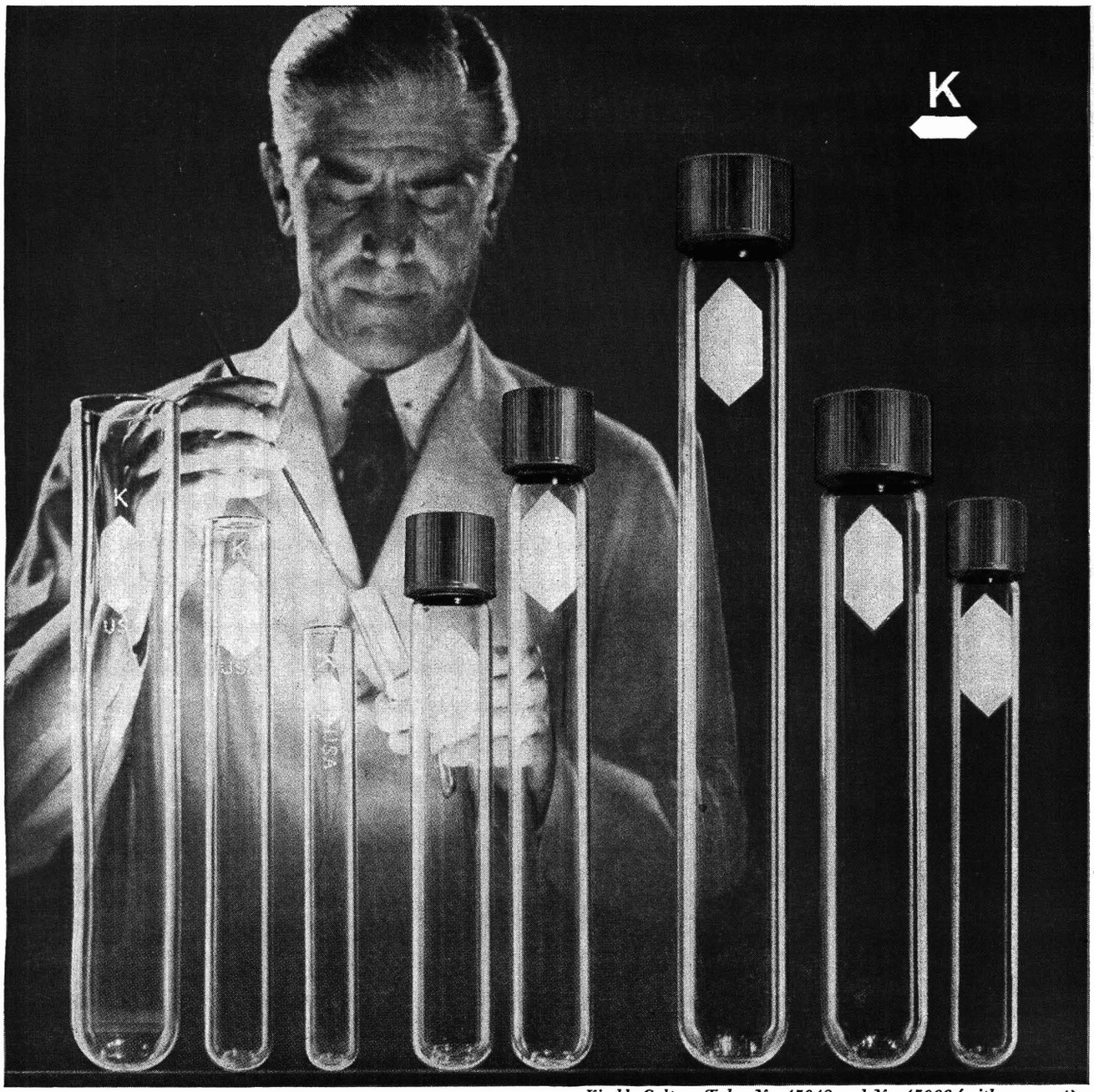

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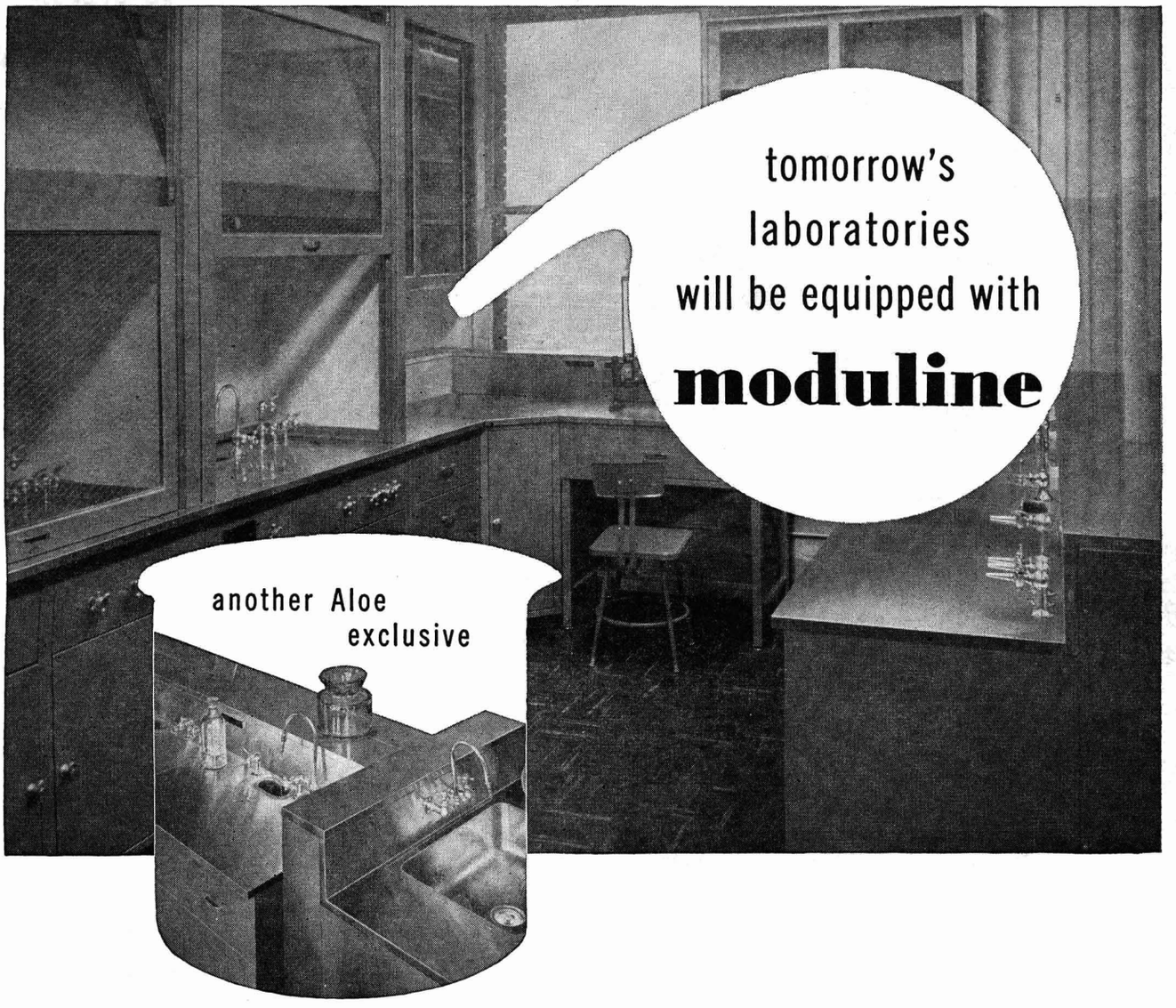

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the analyst's column

THE anniversary meeting of the AMERICAN CHEMICAL SOCIETY next September will afford an unusual opportunity for the members of the Division of Analytical Chemistry to meet a number of foreign chemists and also for these chemists to meet and get acquainted with us. The following men have accepted invitations to take part in the symposia which will continue throughout the New York meeting:

C. J. van Nieuwenburg is a graduate of the Technical University of Delft, Holland. He was, for several years, a research chemist in a Dutch glass factory and some twenty-five years ago he was appointed professor of analytical chemistry in the University of Delft. He has recently been interested in microchemistry, including semimicroqualitative analysis, and for many years he has been chairman of the Commission on New Reagents and Reactions of the International Union. He will be the speaker at the dinner of the Division of Analytical Chemistry, September 4.

Clement Duval, La Sorbonne, Paris, will discuss inorganic analyses making use of infrared spectra and will also tell us about new achievements in automatic gravimetry.

R. C. Chirnside from England will discuss the coordination of analytical methods in industry.

Fritz Feigl, Ministry of Agriculture, Rio de Janeiro, Brazil, is already well known to analytical chemists in this country, having spoken at the Summer Symposium of our division in 1949. He will present a discussion of some phase of microanalysis.

Wolfgang Kirsten, of Uppsala University in Sweden, will also take part in the microchemical symposium.

A number of other European chemists have expressed their intention to be present, either at this meeting or at the Congress the following week. Among them is G. Schwarzenbach of the University of Zurich. He is one of the most outstanding physical and analytical chemists in Switzerland, and his work during the past six years on complex formation between metals and ethylenediamine tetraacetic acid and similar types of acids is of great interest to analytical chemists.

It is expected that a number of colleges and universities will invite these chemists to give lectures. This is a wonderful opportunity for our students and faculties to

(*Continued on page 17A*)

THE ANALYST'S COLUMN

hear these eminent men and to get acquainted with them. It will enable the visitors to become familiar with our laboratories and the research that is now in progress.

It is impossible to overestimate the value of such contacts. Such an arrangement would also pay the expenses of these speakers, none or few of whom could otherwise afford to take extensive tours in this country, no matter how much they might wish to do so. It is hoped that a large number of institutions will cooperate in arranging such tours.

IT IS surprising and discouraging to note how many of the chemists who regularly attend meetings of the Division of Analytical Chemistry, and who often take part in them, are not listed as members. In most cases they have merely neglected to pay the modest dues of $1.00 a year. This division should be one of the largest in the Society, judging by the attendance at its meetings. By joining the division a chemist makes it possible to increase the number of activities in which it can participate. An important one is the Summer Symposium held in June, at which some particular field of analytical chemistry is covered by experts in the field.

The division also assists in the project of formation of local analytical groups within different sections. J. K. Owens, Experiment Station, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del., is chairman of the Committee on Local Groups. A number of such groups regularly hold meetings at which the attendance is high and the discussions are spirited and helpful.

The division has a number of committees working on various projects of interest to analytical chemists, such as, for example, the standardization of microchemical apparatus.

Another advantage in being a member of the division is the receipt of an advance copy of the abstracts of papers to be presented at meetings, and of course a reduction in the price of the book containing the complete abstracts of papers presented before all divisions.

The chairman of the Membership Committee, K. G. Stone, Michigan State College, East Lansing, Mich., would welcome suggestions for reaching those analytical chemists who are not members but should be. If members would send him the names of their acquaintances who might be interested in joining the division, they would be doing a favor both to the division and to the individuals.

H. H. WILLARD

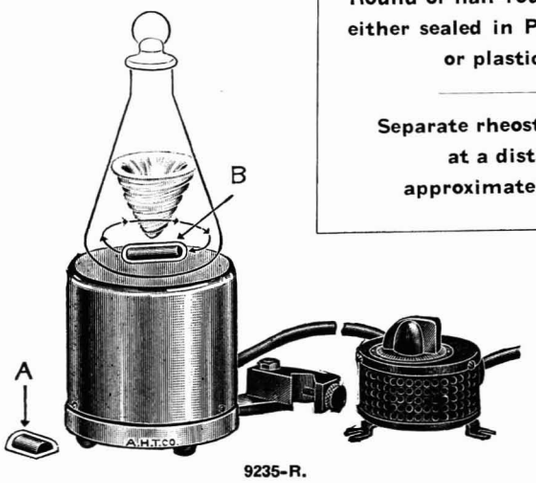
A.H.T. CO. SPECIFICATION

MAGNETIC STIRRING APPARATUS

For variable speed stirring action within *either closed or open vessels*

Round or half-round stirring bars, either sealed in Pyrex brand glass or plastic coated

Separate rheostat for control at a distance of approximately 30 inches



9235-R.

Fig. 1. Showing general stirring in a stoppered flask.

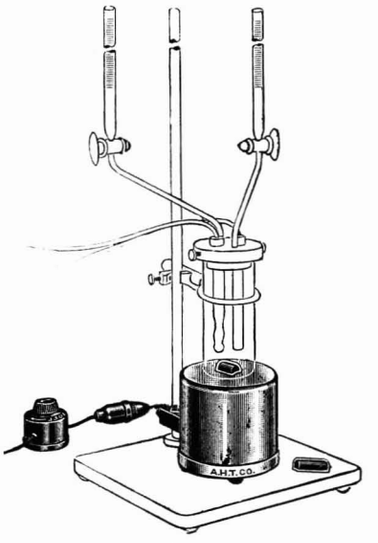


Fig. 2.

Showing stirring in electrometric titrations with air and moisture excluded.

MAGNETIC STIRRING APPARATUS, A.H.T. Co. Specification. A compact, quiet-running apparatus which utilizes a rotating field of magnetic force to induce variable speed stirring action within *either closed or open vessels.* Stirring is accomplished by means of small magnetized bar A or B, which is placed in the liquid to be stirred and which is rotated by magnetic force applied below the container.

Consisting of a permanent bar magnet attached to the shaft of an electric motor and mounted in an aluminum housing with flat top 4¼ inches diameter and 4½ inches high. Can be used either on the table or on a support rod, attached by means of a clamp with swivel joint. Any type of vessel of glass, porcelain or non-magnetic metal, can be used, i.e., flasks, beakers, culture dishes, crystallizing dishes, evaporating dishes, test tubes, weighing bottles, etc. A ring-type burner is suitable when stirring at elevated temperatures. Suitable for any stirring operation which involves 1 ml to 1 liter of liquids with viscosities up to that of a 50% glycerol solution. Particularly convenient for use in closed systems.

9235-R. **Stirring Apparatus, Magnetic, A.H.T. Co. Specification,** as above described, with one each magnetized stirring bars A (small, half-round, plastic coated) and B (large, round, glass coated), ⅞ inch and 1¾ inches long, respectively; rheostat with graduated dial, 8-ft. connecting cord, and directions for use, but without glass vessel. For 115 volts, 50 or 60 cycles, a.c. **38.50**

9235-S. **Ditto,** but without stirring bars **33.40**

9235-T5. **Stirring Bars, Magnetized, Round, Glass Coated,** consisting of a cylindrical permanent magnet sealed in Pyrex brand glass.

Overall dimensions, inches	⅞ x 9/32	1¾ x ⅜
Each	2.65	2.90

9235-U. **Stirring Bars, Magnetized, Half-Round, Plastic Coated,** consisting of half-round permanent magnet with baked on lustrous black plastic coating which was developed to eliminate accidental breakage of glass-enclosed bars. This plastic coating will withstand continuous use in boiling water and has good chemical resistance to weak or strong acids and alkalies except those with oxidizing properties such as nitric acid, alkaline peroxides, etc.; also resistant to organic solvents; overall width ½ inch, overall thickness ⅜ inch.

Overall length, inches	⅞	1⅝
Each	2.55	2.95

9235-U5. **Ditto, Round, Plastic Coated,** generally similar to 9235-U, but with cylindrical permanent magnet.

Overall dimensions, inches	⅞ x 9/32	1½ x ⅜
Each	2.65	2.90

10% discount in lots of 12 or more, 9235-T5, 9235-U and 9235-U5, one size or assorted.

ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

Random Thoughts on the Pittsburgh Conference

THE recent Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy registered new highs in attendance, in the number and quality of the papers, and in the variety of new and interesting apparatus shown by the exhibitors.

We say "well done" to those in the Pittsburgh Section of the AMERICAN CHEMICAL SOCIETY and in the Spectroscopy Society of Pittsburgh responsible for the success of the conference and particularly the cochairmen, H. Freiser and J. J. McGovern.

We doubt that the most optimistic analyst in attendance at the recent conference would have prophesied five years ago that a meeting and exposition of such magnitude could be staged even in Pittsburgh, billed modestly on the front cover of the program as the "Center of Analytical Chemistry."

In reviewing the progress made and the success achieved, let us not forget the early pioneers. With little more than a rugged individualism and belief in the importance of analytical chemistry to guide them, they determined to develop annual conferences in Pittsburgh that would provide real scientific assistance to analysts and focus attention of management on the important role of analytical chemistry in research and production. They have succeeded probably beyond even their own expectations. Today the Pittsburgh conferences are self-rooted and firmly established.

Beginning with the very first conference, it has been a continuing policy to present, in addition to papers of a strictly scientific and technical nature, discussions on various broad problems of interest to the profession of analytical chemistry.

A number of such papers featured in the 1951 program included Robert T. Hall's excellent discourse on industrial analytical research, D. G. Nicholson's up-to-date report on trends in academic analytical chemistry, the businesslike paper by J. W. Stillman and A. H. Hale of Du Pont on the development of an efficient analytical record system, and the interesting description of the role of the analytical chemist in industrial health matters by Dohrman H. Byers. These and other papers of a broad general interest will appear in later issues of ANALYTICAL CHEMISTRY.

Hall presents a very pertinent picture of the analytical chemist that cannot be stressed too often either to management or to the young analytical chemist.

"It is apparent," the author states in describing the Hercules setup, "that these men in the course of their work, must make a rather wide circle of contacts within the company. They necessarily gain a considerable familiarity with many of the research programs, process developments, and technical service activities of all the company's operating departments. This group is perhaps one of the best places in the research organization for acquiring, in a relatively short time, a reasonably good over-all picture of the company's technical activities. This situation leads naturally to a number of possible outlets for the personnel. For example, one of our men has recently progressed into the position of chief chemist at one of our largest plants; others have been transferred to development groups at the plants—or, in some instances, directly into production. Some have moved into supervisory positions in the research divisions of the various operating departments. A few have gone into sales and into liaison groups such as sales research."

Hall in discussing the basic elements necessary for the successful management of an analytical research group listed the following:

> An attitude on the part of management which puts analytical research on the same basis or level as research of any other type. To state it in another way, the emphasis in every case should be on research and not on whether it is physical, organic, or analytical.
>
> Analytical research chemists must be accorded equal status with research chemists in any other line. They should and must be considered as full members of the research team and the opportunities for advancement must be equal to those prevailing in other branches of research.

Hall listed a number of other basic factors which we will pass over now, as his paper will be published later. We do want to refer again to one statement of his—"they should and must be considered as full members of the research team."

One would think that this very basic element would be thoroughly understood and widely practiced by the managements of what are generally considered to be progressive companies, yet we spent more than half an hour at the conference listening to a young but well-known analyst describe tragic delays that have occurred in production operations in a plant of importance to the mobilization effort because this elementary truth had been ignored either deliberately, or because of sheer ignorance. Happily, this example we believe now to be more the exception than the rule.

PAPER CHROMATOGRAPHY
Instruments and Techniques[1]

RALPH H. MÜLLER AND DORIS L. CLEGG
Washington Square College of Arts and Science, New York University, New York 3, N. Y.

These studies are concerned with an attempt to learn more about the phenomena involved in chromatographic separations in paper. Typical techniques are described and some new instrumental approaches are presented. It is shown, by precise semiautomatic methods, how the influence of geometric shape factors can be determined and predicted. The influence of vapor saturation, temperature, and previous history of the paper is established. It is shown that the motion of liquids through the paper can be predicted accurately by a knowledge of their surface tension, viscosity, and density. With these techniques, it should be possible to predict much of the behavior of a given paper with respect to its suitability for chromatographic work and to extend the methods to the kinetic behavior of the individual fractions undergoing separation.

THE use of filter paper as a medium for the separation, identification, and quantitative estimation of organic, inorganic, and biologically important substances is one of the most active fields of modern analysis. The intensive research in paper chromatography is reminiscent of the early triumphs of analytical spectroscopy. The comparative ease with which microgram quantities of extremely complex substances can be separated has accounted for the solution of problems heretofore considered impossible. The technique is so indispensable and results are forthcoming with so little expenditure of time and material that it is not difficult to understand why chromatography is a highly developed art, about which very little fundamental knowledge is available.

This series of investigations was undertaken to learn something about the mechanism of separations in paper media. The difficulty in obtaining a coherent picture of the phenomenon may be appreciated if it is pointed out that the following factors influence the results:

1. Physical and mechanical properties of the paper
2. Dimensions and geometric shape of the paper
3. Chemical properties of the paper with respect to residual impurities and moisture
4. Nature of the eluting liquid, its density, surface tension, and viscosity
5. Temperature at which the separation is effected
6. Vapor saturation and previous history of the paper

In this work an attempt has been made to maintain a balance between that which will be practical and useful to the chromatographer and that which is reasonably fundamental. Although several quantitative and precise principles have been established in these studies, none of these is a "constant of nature," because when one is working with filter paper, it is a system rather than a definite substance with which one must contend. Although filter paper of good quality is very carefully treated to remove impurities and is subjected to painstaking care in fabrication, it is nevertheless capable of considerable improvement before it exhibits the best behavior in chromatographic separations. The primary object of these studies has been to appraise the many factors that influence the flow of liquids through paper, rather than to devise a "recipe" whereby consistent and reproducible chromatographic results can be obtained. This is precisely the information which has been lacking up to the present time, and enough information has been accumulated in these studies to enable conduct of further research in this field in an ordered and logical manner.

[1] First of a series of three papers. See pages 403 and 408.

Although numerous chromatographic separations were made in the early stages of this research, it soon became apparent that it is one thing to duplicate another worker's results, but quite another to devise the proper conditions for a new and untried separation. In order to obtain quantitative information, it was also necessary to develop suitable instruments for the purpose, and it soon became evident that these must be semiautomatic in order to provide sufficient information in a reasonable length of time. In numerous instances, the semiautomatic nature of the data recording meant the difference between vague inference and accurate data. This is the case for highly mobile liquids and very fast papers.

For convenience, the investigation is divided into three parts: (1) instruments and techniques in paper chromatography, (2) geometric factors in paper chromatography, and (3) kinetic studies on the diffusion of liquids through paper.

Despite the relatively large numbers of observations represented by these studies, most of the pressing questions of chromatography remain uninvestigated and unanswered. What has been accomplished is a rather complete understanding of paper formation, the influence of geometric factors, and the kinetics of solvent motion through paper. The influence of temperature, vapor saturation, and surface active impurities is also established. Instrumental developments have provided complete facilities for conventional chromatography as well as for the special needs of these studies.

A review of the status of paper chromatography up to November 1949 (*2*) describes the principal techniques and apparatus commonly used, including numerous conventional instrumental aids such as photometers and densitometers.

INSTRUMENTS AND TECHNIQUES

The essential facts of chromatography may be demonstrated with absurdly simple items—a piece of blackboard chalk, a blotter, or a disk of filter paper. With very little more elaboration, successful separations can be effected. To varying degrees, one may progressively add means for maintaining vapor saturation, constant temperature, and ease of manipulation or the simultaneous treatment of a large number of samples. The same wide range of facilities may be employed to appraise a finished chromatogram. In some cases—i.e., in a simple mixture of dyes—the final pattern may consist of a beautiful set of colored concentric rings and a superficial glance will supply all the available information. In other cases, the full resources of photometry may be necessary in order to get any information at all.

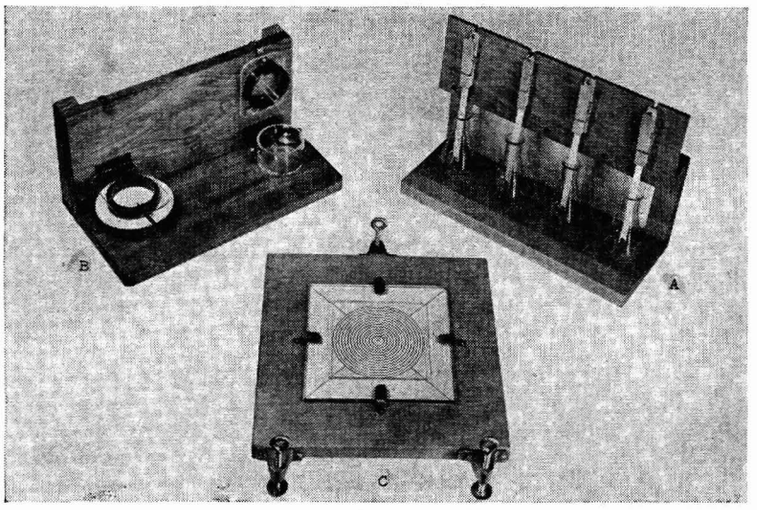


Figure 1. Accessories for Paper Chromatography

Some very simple accessories which are useful in preliminary work in this field are shown in Figure 1.

In the upper right, *A*, is a stand for holding small Erlenmeyer flasks and adjustable wooden clips fashioned from clothespins. A filter paper strip containing a spot of sample mixture can be inserted in the flask containing an eluant, so that the lower edge of the paper is just immersed in the liquid. The rate of rise can be gaged roughly by reference to the coordinates on the rear wall and the degree of separation can be estimated by inspection.

This arrangement is obviously suited only for rough qualitative tests. The principal drawback is the evaporation that occurs at the neck of the flask. This can be avoided by using a cork as support for the strip. If the lower part of the cork is cut away on one half of a diameter, the strip may be held to the vertical section of cork with a small thumbtack or pushpin.

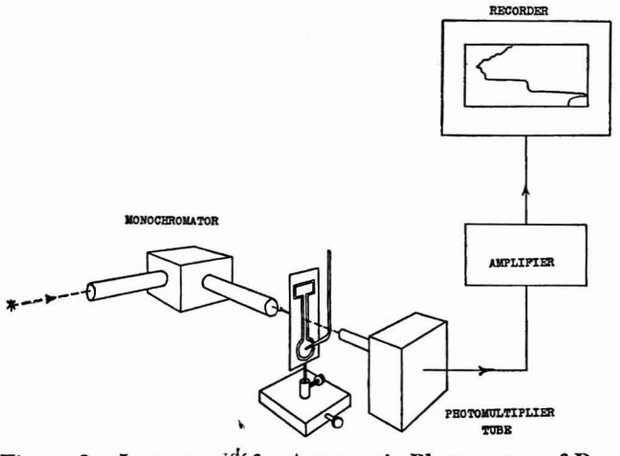


Figure 2. Instrument for Automatic Photometry of Dye Mixtures during Development of Chromatogram

Apparatus for using the Rutter disk technique (*7*) is shown in the upper left of Figure 1, *B*. There is provision for running two samples at once; one in the operating position is shown on the left and another on the right is illustrated in the stand-by position, the better to illustrate the simple construction.

A small crystallizing dish holds the eluting liquid, and this is positioned on the table by thrusting it rearward into a stout V-shaped block of brass. The Rutter disk with its radial tab is placed on the edge of the dish with the tab dipping into the liquid. As soon as it is in position, the lid is lowered and the elution can proceed in an essentially vapor-tight enclosure. The lid consists of a stout brass ring, the medial diameter of which is the same as the glass dish. It is fastened from the top to a sheet of polystyrene which is hinged at the rear to the back wall. The entire assembly is readily loaded and unloaded and can be cleaned and set up for a new run in a short time. It is considerably more stable than a stack of two dishes or watch glasses as ordinarily used in the Rutter technique.

Typical results obtained by this technique are shown later in this paper.

The device shown in *C*, Figure 1, is very convenient for rapid tests on papers or powdered adsorbents.

The baseboard is readily leveled by means of the three wing screws, and on it is mounted a heavy sheet of plate glass under which a pattern of concentric rings is drawn for the purpose of centering a filter paper disk. The disk is covered with another sheet of glass with a hole bored in its center. Through this, a sample can be admitted, followed by a suitable eluting liquid. This arrangement has been recommended (*8*) for preliminary examination of powdered adsorbents such as alumina, talc, sugar, and starch.

One of the earliest instruments to be developed in these studies permits one to photometer the progress of separations continuously and in microgram quantities. The essentials of this instrument are shown in Figure 2. Complete details of this method have been published (*3*, *4*), and typical results shown in Figure 3 are reproduced here merely to relate these developments to the general instrumental approach. Although this instrument was the first to be described in the literature for dynamic studies in paper chromatography, it has outlived its usefulness in this laboratory as a means of obtaining more detailed kinetic information. For its original purpose, it still possesses considerable analytical utility in the assay of mixtures.

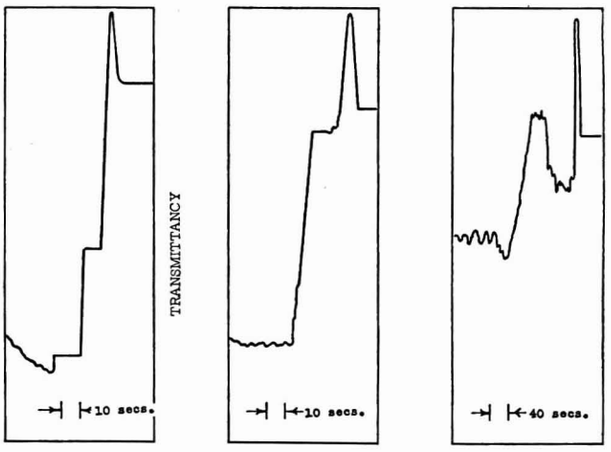


Figure 3. Automatic Photometry of Dye Separation during Development of Chromatogram

Left. **Aniline orange and malachite green**
Center. **Congo red and malachite green**
Right. **Eosin and methylene blue**

REFLECTANCE DENSITOMETER

Several investigators have recommended the examination of paper chromatograms by a densitometer of some sort (*1*, *2*). In this way one can estimate the intensity of color as a function of distance along the chromatogram and gain more quantitative information about the distribution of the various substances.

As a rule, some commercial form of photoelectric photometer has been used, and an attachment has been provided whereby the paper strip can be pulled through the instrument step by step and its transmittancy measured at convenient intervals. The results can then be plotted as a function of distance. This is laborious, and unless a large number of measurements are made, the results are unlikely to be worth the trouble.

For the rapid examination of finished chromatograms, the very simple, completely automatic reflectance densitometer shown in Figure 4 has been assembled from readily available components.

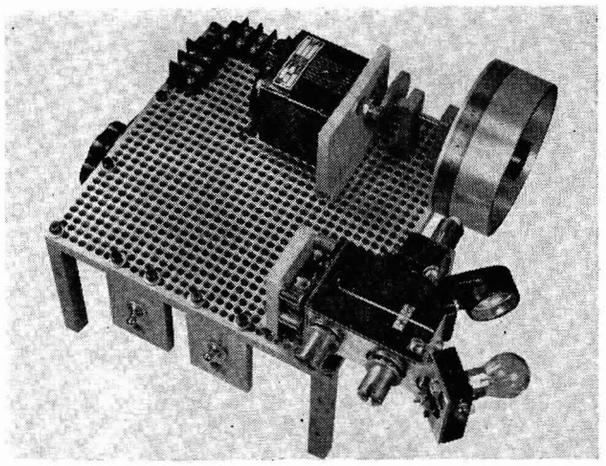


Figure 4. Reflectance Densitometer for Completed Chromatograms

The mechanical parts, such as the base plate, legs, ball-bearing pedestals, shafting, and gears, were obtained from Servomechanisms, Inc. A large aluminum drum with a flush-mounted steel millimeter scale is used as a specimen holder. A smaller drum, also with scale, is available for very small specimens.

Through a five-to-one gear reduction, the scanning drum is rotated at a uniform rate by the 1 r.p.m. Bodine synchronous motor. Gear ratios are readily changed for lower or higher speeds. A photoelectric pickup unit, which appears in the right foreground of the photograph, is the scanning head built by Times Facsimile, Inc. It consists of a 6-volt lamp, the light from which is focused on the periphery of the drum by a condensing lens. Light reflected from an extremely small spot of the illuminated area is picked up by the microscope objective and brought to a focus on a photocell. The original facsimile scanner contained a special frequency-modulated phototube, but for circuit simplicity and ease of matching to a recorder, this was replaced by a small barrier-layer photocell. The output of this cell is connected directly to a 100-ohm General Radio potentiometer (voltage divider), and the slider and one end of the potentiometer are connected through shielded and grounded wires to the Brown Electronik recording potentiometer. Thus the output of the photocell can be varied from zero to maximum.

A filter holder (not shown in photograph) is mounted over the lens barrel, and a Corning filter best suited to the photometric problem in hand can be inserted at this point. The entire densitometer is fastened to the laboratory wall with the aid of two pipe flanges and a short nipple. A small constant-voltage transformer and line switch are located next to the densitometer. The scanning drive motor is provided with a start-stop and reversing switch. The entire instrument can be set in operation within a few seconds.

Specimens to be scanned are cut from the chromatogram, if necessary, and wrapped around the drum, and the ends are affixed by Scotch tape. By reference to the adjacent millimeter scale, strategic points on the chromatogram can be identified for later correlation with the densitometer record.

In some respects, it is more convenient to draw sharp pencil lines across the chromatogram to locate original sample site, end of the chromatogram, etc., because the photometer will read these and write sharp inflections on the chart. Too many of these identifying lines, or their injudicious location, will confuse the record. An alternative consists in pasting a very narrow strip of aluminum foil at the point of identification. As a result of its high reflectivity, the aluminum marker will produce upscale deflections, whereas most parts of the chromatogram give uniform or decreasing reflectance. Mounting operations are best conducted at a desk or table, after which the drum is easily slipped over the end of the drive shaft, rotated to the start line, and locked in position with a small spanner wrench. No focusing operations are required because the sample is flood-lighted and the receiving objective is in fixed focus.

As the sample drum rotates uniformly, the photocurrent varies in proportion to the reflectivity and this is faithfully reproduced on the recorder chart. As the latter is also synchronously driven, there will be a 1 to 1 correspondence along the displacement axis. There has been no occasion to correct any records in terms of self-calibration pencil marks, and strict synchronism can be relied upon. One advantage of this instrument resides in the fact that it will repeat readings indefinitely without attention. Examples of its performance are shown in the following illustrations, wherein the Rutter technique is discussed more fully.

In an optical sense, reflectivity measurements have very limited use. Reflectance is a very complex function of concentration; indeed, no exact relationship is known. Although provision is readily made for using a plastic or glass drum and slightly modifying the optics of the system in order to get transmittancy values, the present scheme has been retained for its great utility in respects other than concentration.

RUTTER TECHNIQUE

Two examples are given to show what can be achieved by the filter paper technique first proposed by Rutter (7). Some of the refinements just described have been applied to it, but these do not detract from the beautiful simplicity of his method.

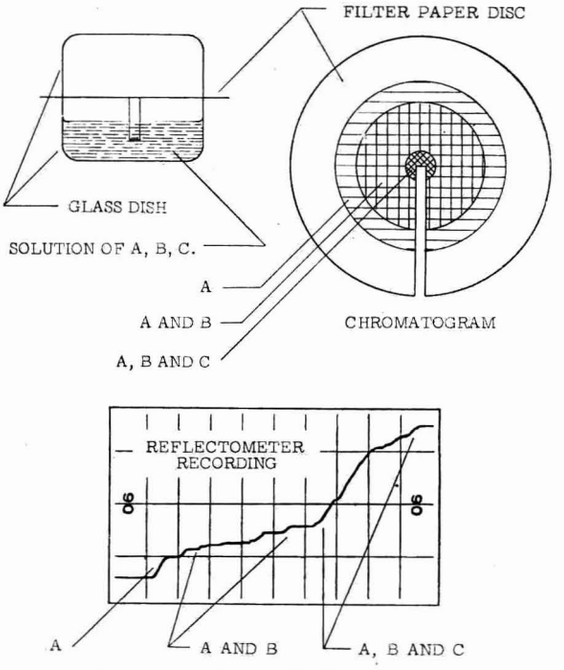


Figure 5. Rutter Disk Technique

All components present in original solution

As shown schematically in Figure 5, the Rutter disk is prepared by cutting a radial strip into a circular filter paper disk. The strip is bent at the center so that it projects perpendicular to the plane of the disk. About half of it is cut off. This is the disk which is now mounted between the edges of two crystallizing dishes, as shown in the upper left.

Consider, first, a case not originally described by Rutter, but for which the technique is well suited. The lower dish contains a solution of three dyes—red, lavender, and blue. These happen to be the constituents of Wright's stain, which is essentiall

eosin and methylene blue with the lavender as an impurity, though azure blue is often added as a third component. As the disk is placed in position with the radial tab dipping into the solution, liquid will rapidly rise in the strip and then spread in circular fashion in the main disk. Close inspection will reveal that pure solvent spreads first, soon to be followed by eosin and this in turn by a mixture of eosin plus the lavender dye, and the third or last zone contains all three dyes and, of course, solvent. After considerable spreading has occurred, the process is stopped and the paper is removed and air-dried. If a diametral strip is cut from this chromatogram and mounted on the densitometer drum, the reflectometer record will show a stepwise pattern for the three respective zones. These are illustrated schematically and idealized in the upper right of Figure 5.

What has occurred here is that all solutes are adsorbed on the paper and only pure solvent gets through. A point is soon reached, however, in which the paper becomes saturated in the least adsorbable component, eosin, and breakthrough occurs. Presently, the same portions of the paper become saturated with lavender dye and this breaks through, but accompanied by more eosin which is continually supplied by the reservoir. Ultimately, the most strongly adsorbed methylene blue will also break through, and for this part of the paper all three components will move unadsorbed.

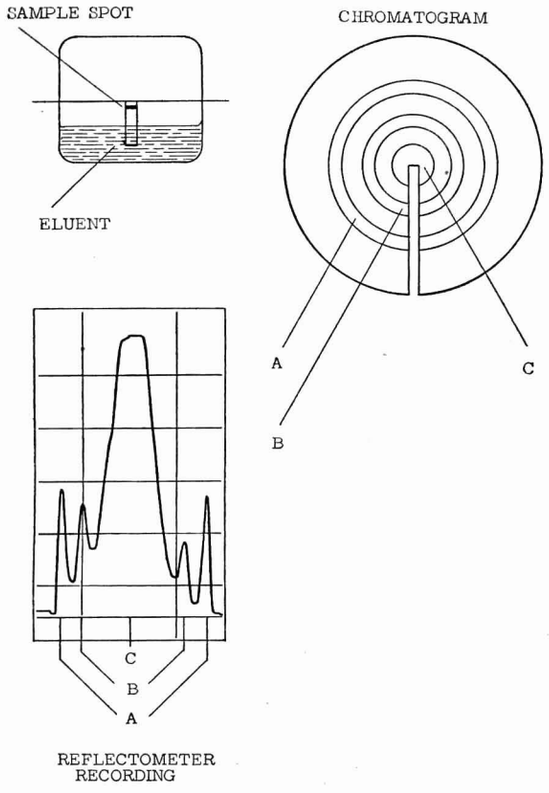


Figure 6. Rutter Disk Technique

Components of sample progressively eluted by suitable solvent

The corresponding case for columns has been reported recently by Nestler and Cassidy (*6*) in determining the adsorption isotherms of low molecular weight fatty acids on charcoal. Here, the principle of successive breakthrough can be utilized to obtain conventional adsorption data.

The more conventional application of the Rutter technique is illustrated in Figure 6, in which the sample is not in solution but is deposited in a concentrated spot on the center of the disk, or as shown here, just below this point on the radial tab.

A suitable eluting liquid is placed in the lower dish and allowed to rise in the pendant tab. Preferential elution of the dye mixture then begins, and eventually each component is separated from its neighbors as a distinct ring. Schematically the state of affairs is shown in the upper right of Figure 6. If a diametral strip is now cut from this chromatogram and measured with the reflectance densitometer, the individual zones are sharply defined, as shown in the lower left.

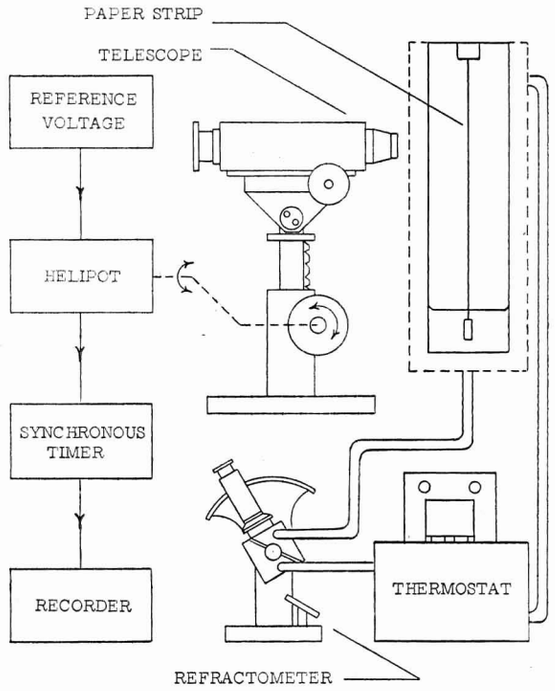


Figure 7. Semiautomatic Instrument for Kinetic Measurements

This recording illustrates the advantage of scanning across a diameter, because then the pattern is measured twice and any lack of symmetry is at once detected. Indeed, such is the case in Figure 6, and the degree of asymmetry is a measure of the slight inhomogeneity of the paper. More is said about this factor in the following paper.

Regardless of which technique is used, the Rutter disk method is extremely useful. When combined with the densitometer, it enables one to evaluate procedures, choice of eluants, etc., in rapid and exact fashion. In the second variation, wherein distinct zones are produced, one can perform very exact quantitative determinations by simply cutting out the separate zones with a pair of scissors, extracting each piece completely with an appropriate solvent, and then measuring the combined washings in a spectrophotometer.

The relative motion of solvent and the various zones as a function of time has been established (*5*), and the radial spreading of both solvent and dye follows a square law such that the square of the radius is directly proportional to elapsed time. These relationships enable one to arrive at a dynamic estimate of R_F values. For this purpose, the automatic reflectance densitometer measurements are ideally suited.

INSTRUMENT FOR KINETIC STUDIES

Most of the equipment, so far described, has provided the chromatographer with more precise and elegant tools for his work. When attempts were made to introduce a greater degree of predictability into chromatographic work, it became apparent that too little was known about the motion of solvents through paper. With this initial ignorance, it would seem hopeless to attempt to explain the more complex case of the relative motion of solutes.

The principal emphasis in these studies is, therefore, on the establishment of the exact nature of solvent motion through

paper. This is now certain enough so that further work may proceed in logical fashion.

The schematic diagram of Figure 7 shows the essential features of semiautomatic equipment for kinetic studies of this sort.

A jacketed chamber, held at constant temperature, holds the paper strip which can be brought to equilibrium with the saturated vapor of the liquid to be investigated. When this state is attained, the liquid in question can be admitted to the chamber in fixed amount such that the pendant strip becomes immersed in it to a fixed depth. As the liquid rises in the strip, its motion is followed by the operator with the traveling telescope.

It is almost impossible for the operator to follow this motion and record the position and the elapsed time with sufficient precision. More correctly stated, one can obtain far more precise data in a given time if the operator is relieved of certain details during the run. This was achieved by coupling the elevating knob of the traveling telescope with a precision helical potentiometer (Helipot). The position of the slider of the Helipot is, therefore, at all times a measure of the height of the telescope. The total voltage impressed across the Helipot is chosen from a reference voltage source of high constancy, and its magnitude can be chosen so that the maximum travel of the telescope will produce any desired deflection on the graphic recorder. For convenience, the choice is such that one division on the recorder chart (about 0.125 inch) corresponds exactly to 1-mm. travel of the telescope.

The Brown Electronik recorder used in this work gives full-scale deflection (100 divisions) for an input signal of 2.5 mv. The maximal travel of the telescope is 72.4 mm. Therefore, the reference source is adjusted to provide enough potential to produce a net recorder deflection of 72.4 divisions. For good mechanical reasons, it is undesirable to use a recorder zero corresponding to zero on the chart, because the pen cannot move below zero, and any negative drift in the signals would escape undetected. Therefore, the voltage reference source provides a constant reference voltage, with vernier adjustment, to bias the recorder to an arbitrary "zero" of 20 divisions. Any motion of the telescope above its base position (20 divisions) then causes a deflection of one chart division per millimeter of travel with a maximum deflection of 92.4 divisions (72.4 + 20). All recorded data are, therefore, diminished by 20 to give the net distance in millimeters. This arithmetical chore can be eliminated in a number of ways and this would have to be done if the instrument were designed for large scale use.

For runs of any considerable length, it would be excessively fatiguing for the operator to keep the telescope continuously focused on the liquid boundary. It was, therefore, decided to make the recording intermittent and, as the block diagram illustrates, a two-position cycling timer serves to connect the Helipot to the recorder once every minute and to hold it in the writing position for exactly 5 seconds. In addition, it flashes a red warning light to the operator, so that a last-minute adjustment of the telescope may be made. Although this signal is essentially a "hands-off" warning to the operator, it still permits him to make a final correction in telescope position, provided he stops such adjustments before the end of the 5-second interval. The 5-second interval is ample for the operator and safely within the response time of the recorder, which is something less than four seconds for full-scale deflection. As the timer is synchronously driven, the precision in timing is more than adequate and one has a constant cross check on the chart, which is also driven by a synchronous motor.

Figure 7 also indicates that a thermostat pumps constant temperature water through the observation chamber and also through an Abbe refractometer.

A more detailed view of the observation chamber is shown in Figure 8.

It is made of heavy sheet brass with the central chamber open at the top. Large sealed chambers, through which constant temperature water is pumped at high speed, are at the left and right and on the bottom. Near the top a brass pipe connects the chamber on the left with the one on the right, and the flow of constant temperature water through the system is clockwise as shown. The interior of the chamber is a dull black for good heat exchange and is produced by chemical oxidation of the brass case (immersion in copper carbonate dissolved in ammonium hydroxide). This finish is insoluble in all liquids studied. Glass windows are mounted on the front and back of the chamber for viewing the strip. In use, the entire chamber is brought under a head which holds the accessories and then raised into position, where it is retained by a simple jack.

The head is a wooden frame from which a steel rod descends, on a threaded portion of which a sample clamp is mounted. The paper strip can be inserted between the smooth jaws of this clamp and small setscrews can be tightened to retain the strip. The paper is held reasonably straight by a small brass paper clip

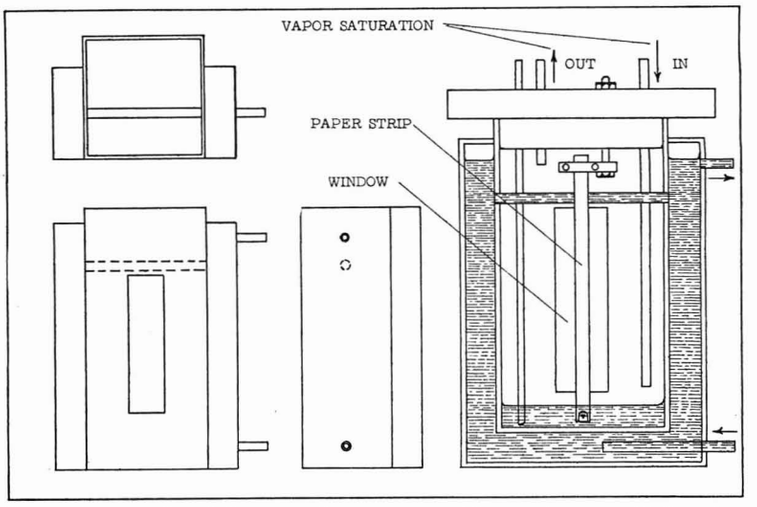


Figure 8. Details of Constant Temperature Vapor-Saturated Chamber for Holding Paper Strips

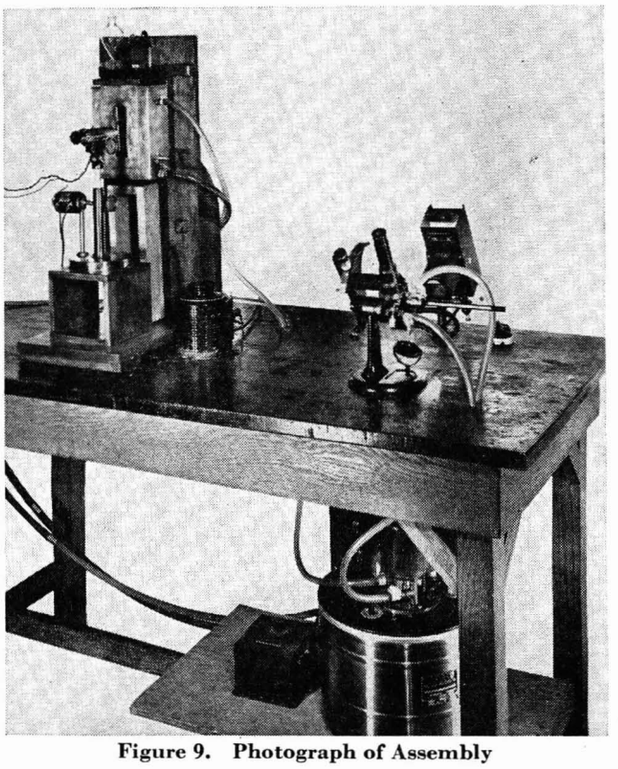
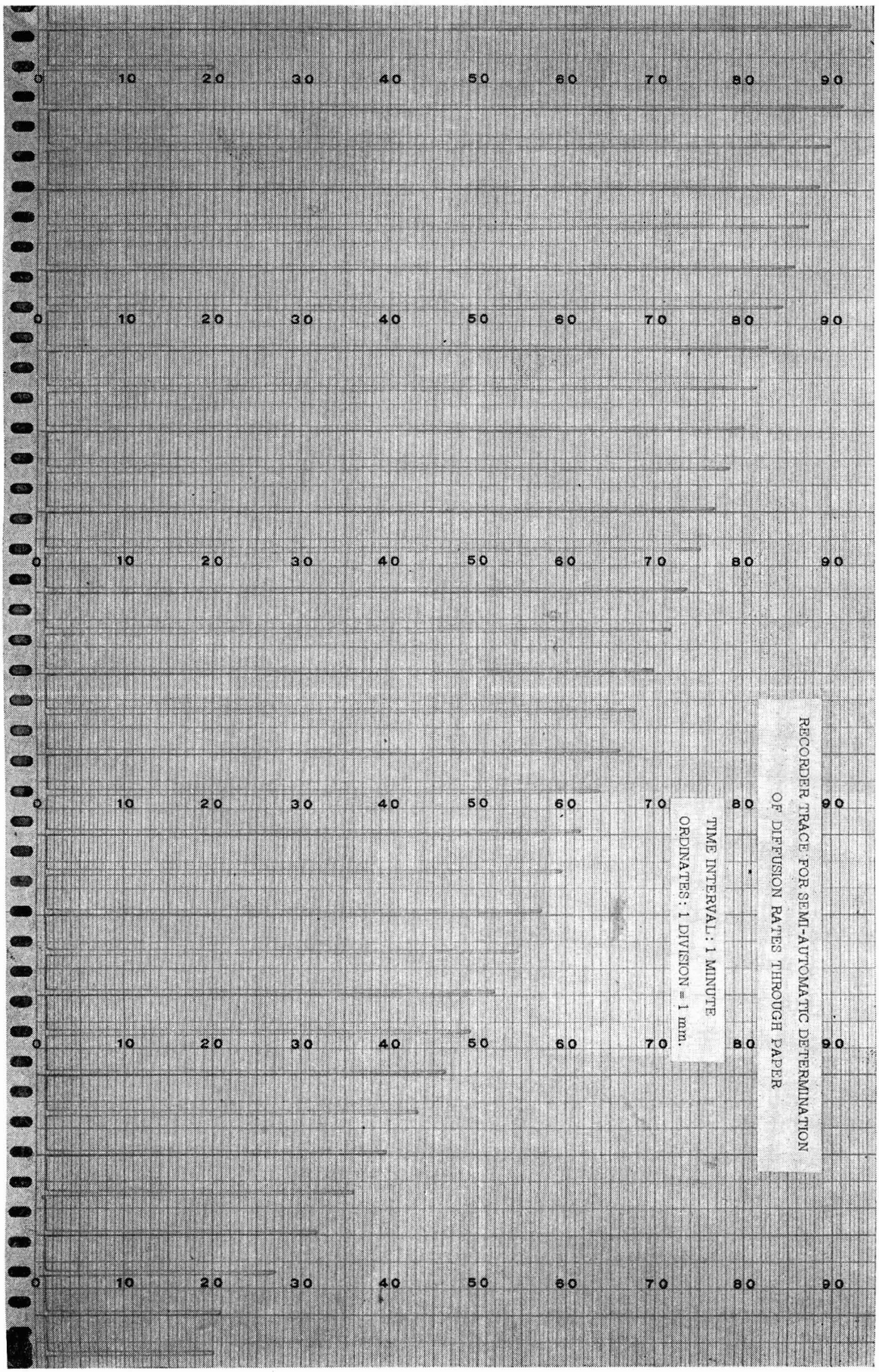


Figure 9. Photograph of Assembly

Figure 10. Recorder Trace for Semiautomatic Determination of Diffusion Rates through Paper



RECORDER TRACE FOR SEMI-AUTOMATIC DETERMINATION OF DIFFUSION RATES THROUGH PAPER

TIME INTERVAL: 1 MINUTE
ORDINATES: 1 DIVISION = 1 mm.

affixed to its lower end. The paper clips are subjected to four rinses with acetone to remove lacquer and dried over a steam radiator. Inlet and outlet tubes are provided to circulate vapor. Before each run, tank nitrogen is bubbled through a fritted-glass washing bottle containing the liquid under examination and then circulated through the chamber holding the paper strip. A dropping funnel is also held by the head, from which the liquid can be admitted when a run is begun. A Weston dial thermometer is also mounted on the head, but this is used only for rough indications of chamber temperature and will not indicate the degree of regulation maintained by the thermostat, which is about ±0.02° C. A 40-watt tubular incandescent lamp illuminates the chamber through the rear window and its brilliance can be controlled over a wide range with a Variac.

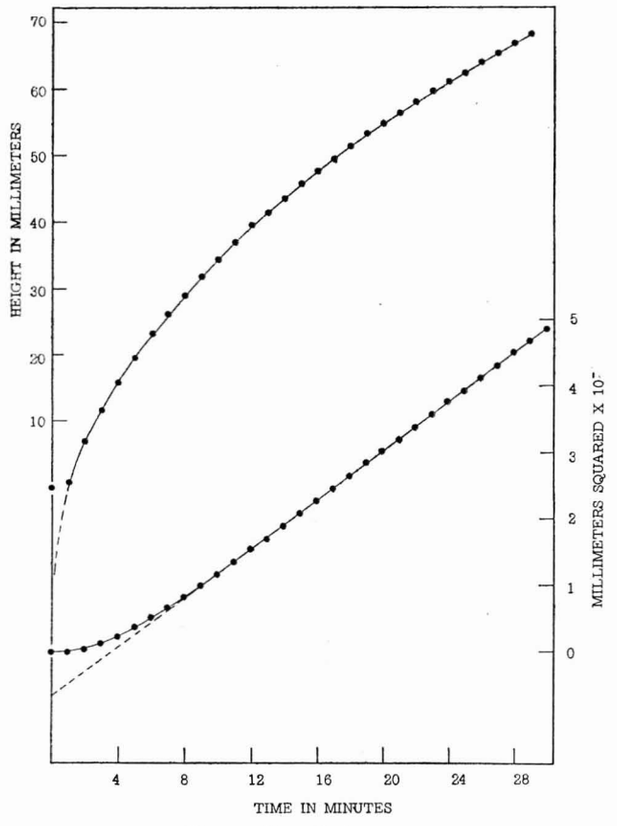


Figure 11. Deflections

Figure 9 is a photograph of the assembly showing thermostat, refractometer with its light source, and the kinetics apparatus. The timing mechanism and Brown recorder are not shown.

A typical recording is shown in Figure 10. Elapsed time reads from right to left, and the pen excursions for each 1-minute interval can be seen to rise progressively during the run. The general parabolic contour is clearly evident, and the net deflections are reproduced graphically in Figure 11. In this graph, the net heights in millimeters are plotted as ordinates against elapsed time in minutes. In the second plot, the square of the height is plotted against time which, beyond an initial interval, is linear. As is shown in the following papers, the individual readings can be recalculated from a general square-law equation within the precision of measurements, which is ±0.10 mm.

This instrument enables one to collect diffusion rate data with ease, speed, and good precision. Numerous cases were encountered in which the rates were so high that the entire phenomenon was over before three 1-minute writing intervals had elapsed. These were handled by the same instrument by simply locking the automatic timer in the writing position and moving the telescope continuously along the advancing liquid boundary. A typical example of continuous tracking is shown in Figure 12, along with a plot of the data according to the square law. Even for such very fast diffusion, the data are not greatly inferior to the conventional rates. A few abrupt discontinuities in the recorder curve can be seen. These actually represent a lag in wrist movement as the operator sought a new grip on the elevation knob, but in no wise affect the average shape of the curve.

Aided-tracking schemes are well known, especially in military range-finding equipment, whereby an operator regulates the speed of motor drives to keep a cross hair on a target; so, inherently, there is no particular limit on speed in such cases. Refinements of this sort were not necessary, and, in any case, completely automatic scanning is not only possible but already partly constructed for extensions of this work.

Since this work was completed, a data printer, supplied by Taller and Cooper, Inc., according to the authors' specifications, has been installed, which greatly simplifies measurements and affords a considerably cheaper assembly. This is a five-digit counter, the instantaneous reading of which is automatically printed on common paper tape at 15-, 30-, 60-, or 120-second intervals, as desired. A manual button is also provided with which the count can be printed at any desired time. The use of this data printer may be understood by referring to Figures 7 and 9.

The coupling between the telescope drive shaft and the Helipot is removed and the telescope is coupled to the print wheel shaft through the intermediary of a 1 to 25 step-up gear train. The print wheels thus assume a value which at all times is proportional to the telescope position and can be printed at any one of the desired intervals. A warning signal for the operator is also provided by an advanced position microswitch on the timing cam. As a consequence of odd values in the pitch of the telescope drive, no even or integral correspondence between digit values on the printer and linear position of the telescope drive was attempted,

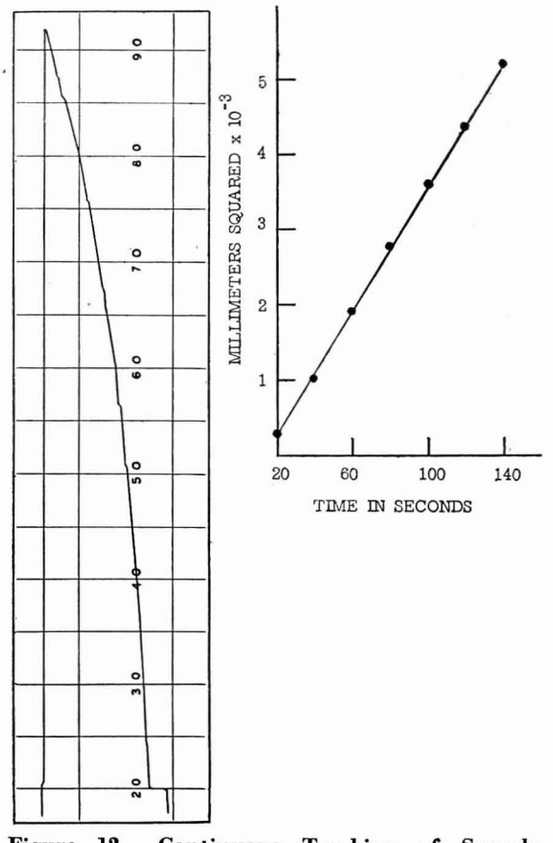


Figure 12. Continuous Tracking of Sample through Fast Paper

but with the 1 to 25 ratio, each recorded digit corresponds to less than 0.1 mm. of travel. A simple correction factor is applied to the final computed slopes.

Aside from reduced cost, one striking advantage of the data printer over the recording potentiometer is the fact that one does not have to reconvert deflections into numbers—these are printed at exact time intervals and can be used directly to compute slopes in the quadratic equation.

An alternative use of the data printer has not been used in this work, but is suited for an earlier method (*5*), in which the print wheels are driven continuously by a small synchronous motor at 60 r.p.m. The telescope is not used for displacement measurement, but merely to view the paper, which in this case has a pattern of pinpoints of light projected on it, spaced in a square-law sequence. Under these circumstances, the liquid boundary will cross successive target positions in equal intervals of time and these can be printed by the observer to the nearest 0.1 second by pressing the manual print control button.

Further uses of these instruments are described in succeeding papers. At this point one may well ask what justification there is for the use of relatively elaborate equipment in studying the motion of liquids and solutes through paper. The authors' experience has more than justified the time and expense involved and, indeed, indicated the utility of still more elaborate and automatic equipment. With what has been described here, it has been possible to accumulate an embarrassingly voluminous amount of data, but in this field, the practical applications of which are almost wholly empirical and the theoretical aspects of which have been highly speculative, there seems to be ample room for precise data.

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Physical and Geometric Factors

BECAUSE paper is used in many ways and under conditions in which its shape, texture, and the admission of liquids vary greatly, a study of some of these factors would seem to be the first order of business in elucidating chromatographic behavior.

With respect to structure, the average piece of filter paper is not merely a heterogeneous assembly of cellulose fibers, because in most cases there is a certain degree of orientation of the fibers. This arises from motion through the Fourdrinier machine, in which the suspension of fibers is felted into a cohesive mat. This preferred orientation is referred to as paper "formation," and the axis toward which the fibers tend is referred to as the machine direction and corresponds to the longitudinal travel of the main screen of the machine.

Formation or preferred orientation is readily demonstrated and susceptible to measurement to about 1% by admitting a dilute dye solution to the paper from a very fine capillary. If the delivery is slow enough to avoid flooding, elliptical spots will be formed. For the examples shown in Figure 1, the ratio of major to minor axis is a constant and equals 1.18 ± 0.014, independent of the size of the ellipse. It is also evident that the major axes are all inclined more or less in the same direction. For a sample of paper in which this ratio is unity—i.e., where circular spots are formed, there is no preferred direction and its chromatographic behavior would be normal.

Frequently, one encounters specific directions in the literature of chromatography wherein the author states that all separations are best conducted by elution in a direction perpendicular to the machine direction. In the light of the above facts, this is equivalent to saying that the direction in which the rate of flow is the least (along the minor axis) is productive of good separations. Although this is a matter of empirical observation and a useful guide, it by no means follows that a slower rate is the exclusive criterion of good separations.

The upper part of Figure 1 shows how the width of the feeder strip in a Rutter disk influences the rate of flow. In this case, two feeder strips were cut in the same disk, one 2 mm. in width, the other 4 mm. Both feeders were immersed in the same dye solution and removed simultaneously after a suitable interval. The two spots are elliptical in shape, again indicating appreciable paper asymmetry. The respective areas were carefully measured

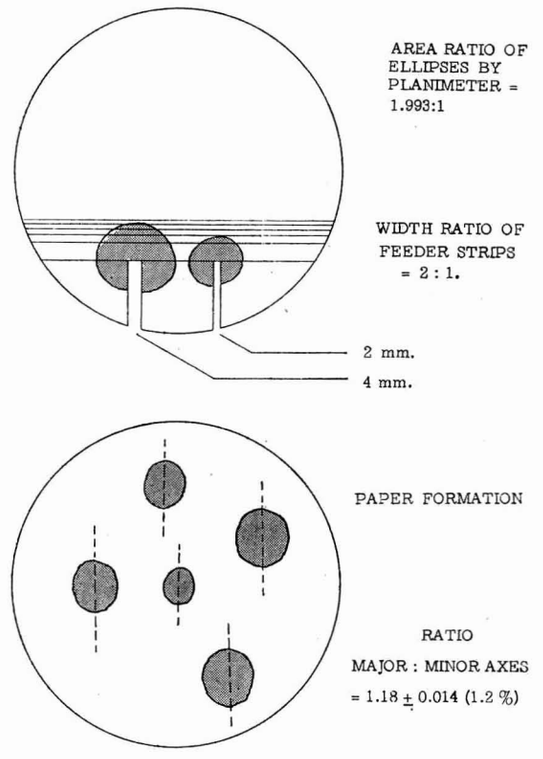

Figure 1. Structure of Filter Paper

with a planimeter and stood in the ratio of 1.993 to 1.000. This agrees within 0.36% with the ratio of feed strip widths. This behavior is in complete accord with the general theory developed in these studies for the rate of flow as a function of dimensional changes. It is referred to later after some additional facts are described. At present, it seems best to discuss the implications of the above behavior.

Because the time of exposure was the same in both cases and the areas stand in the ratio of 2 to 1, it is evident that the respective volumes of solution in the two spots are:

$$V_1 = \pi a_1 b_1 t f$$

$$V_2 = \pi a_2 b_2 t f$$

where $V_1 = 2V_2$
a = major axis of ellipse
b = minor axis of ellipse
t = thickness of paper
f = accommodation coefficient

This is true because the area of an ellipse equals πab and f denotes a measure of the free space in the paper which can be occupied by liquid. It is now evident that in the given time interval twice as much solution must have passed through the larger feeder strip as through the smaller strip. Thus in feeding the large disk with dye solution, we must say that each strip provides an accessibility factor which is proportional to its width.

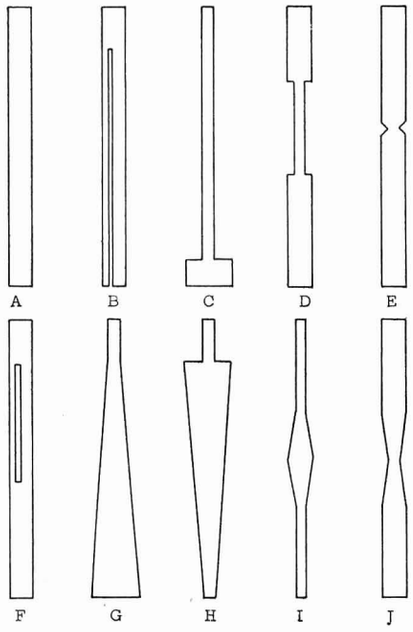

Figure 2. Shapes Used to Study Effect of Geometric Factors on Rate of Diffusion of Liquids through Paper

A more general treatment shows that the effect of geometric factors may be explained completely and quantitatively by assuming that the rate of flow is controlled by two factors: (1) accessibility, as controlled by the dimensions of the feeder, and (2) capillarity, as controlled by the dimensions of the succeeding portions of the system.

This is investigated more exhaustively in the geometric patterns illustrated in Figure 2.

Before any quantitative study of geometric or shape factors could be made, it was necessary to establish some relationship between motion of the liquid through the paper and time. Empirically, one may use the fact that the square of the distance (height of rise, or radial spreading) is directly proportional to time (*2*). This relationship is accurate and reproducible and provides rates well within the experimental precision which, in itself, is high. Actually, this empirical rule represents a special limiting case of the general law governing flow through the paper, and those relationships are discussed in a subsequent paper in this series.

With more than adequate precision we may write:

$$h^2 = Dt - b \qquad (1)$$

where h = the vertical rise of liquid in millimeters (or radial spread in a Rutter disk)
t = time in minutes
D = constant for a given paper and liquid
b = constant and equivalent to an h_0 term

This expression requires some explanation, in that its form arises from the fact that the square-law behavior sets in after an initial time during which the inrush of liquid is unordered and chaotic. This is no plausible assumption; it may be observed directly in the traveling microscope. Experimentally, it is also necessary to observe h and t at some arbitrary time after the admission of liquid to the paper, after which the motion becomes uniform and follows this expression exactly, even for hours for very slow papers.

Under all conditions obtaining in this work, this empirical expression is a precise measure of the results and the slope factor, D, has the dimensions of a diffusion coefficient. As will be shown later, D for a given paper can be identified directly with three properties of the liquid: its surface tension, viscosity, and density. The obvious limitation of Equation 1 is the fact that it predicts infinite height of rise in infinite time. An equation has been derived which is not subject to this limitation, but it is more awkward to use and, for the limited range over which these measurements extend, the two expressions are indistinguishable. This point is more fully discussed in a succeeding paper of this series.

The effect of shape factors has been studied fairly exhaustively with the various shapes shown in Figure 2. From these studies, it may be said that the rate of diffusion is independent of dimension and is affected only by a change in dimension.

For example, if D is determined for the rise of liquid in a narrow rectangular strip such as A, it will be found to be independent of the width. To avoid any complications of strict reproducibility of conditions, this is best shown by using a strip of the shape shown in B. Here a rectangular strip 10 mm. wide is cut to provide two pendant strips of respective widths 3 and 5 mm. The rate of rise in both strips is recorded simultaneously. Because identical conditions prevail, the equality of the D values may be taken as ample proof that the rate is independent of width (Table I).

Consider, now, the case of strip D, in which the rise occurs initially in a width of 10 mm. and then abruptly enters a region of half this width (5 mm.). After an intermediate zone in which the liquid is perceptible swirling about, the rate settles down to a new value which is higher than before. Plotted results are shown in Figure 3.

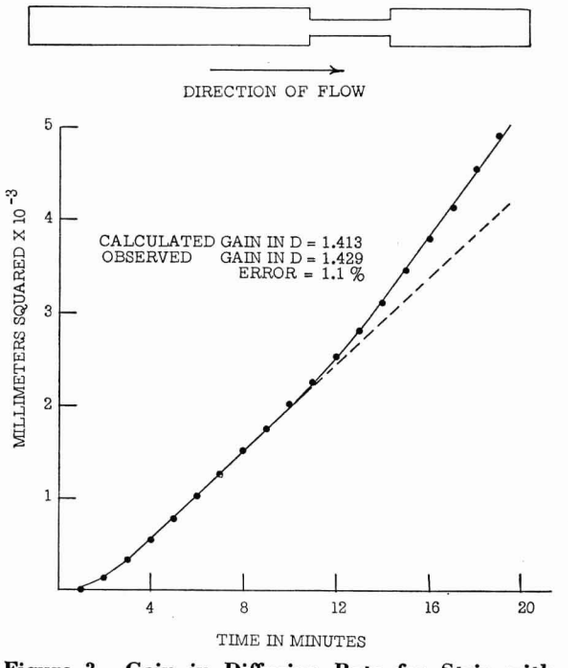

Figure 3. Gain in Diffusion Rate for Strip with Abrupt Dimensional Change

Table I. Effect of Width of Strip

S. & S. paper 598 YD
3- and 5-mm. rectangular strips, Type *B*, Figure 2
Temperature 25° C.
Eluant. Ethyl alcohol–water 84.6%
$n_D^{25} = 1.3630$

Time, Min.	Net Rise, Mm.	h^2	Time, Min.	Net Rise, Mm.	h^2
1	9.8	96	0	0.0	000
3	26.1	681	2	18.9	357
5	36.0	1297	4	31.5	992
7	44.1	1945	6	40.3	1624
9	50.6	2550	8	47.4	2247
11	56.5	3192	10	53.4	2852
13	61.9	3832	12	58.7	3446
15	66.9	4476	14	63.9	4083
17	71.4	5098	16	68.4	4679
			18	72.0	5184

Width = 5 mm. D = 315 mm.²/min.
Width = 3 mm. D = 314 mm.²/min.

Table II. Effect of Shape of Strip

S. & S. 598 ST
Type *D*, Figure 2
Eluant. Ethyl alcohol–water 84.6%
Temperature 25° C.
$n_D^{25} = 1.3630$

Time, Min.	Net Rise, Mm.	h^2
0	0.0	0.0
1	3.2	10.24
2	12.0	144.0
3	18.5	342.3
4	23.7	561.7
5	28.2	795.2
6	31.9	1017.6
7	35.6	1267.4
8	38.8	1505.4
9	42.0	1764.0
10	45.0	2025.0
11	47.6	2265.8
12	50.3	2530.1
13	53.0	2809.0
14	55.8	3113.6
15	59.0	3481.0
16	61.7	3806.9
17	64.3	4134.5
18	67.6	4569.8
19	70.2	4928.0

D_1 = 246 mm.²/min.
D_2 = 351 mm.²/min.

In this and all other variations, it may be shown that the gain, G, in diffusion rate is predicted by

$$G = \frac{1 + R^2}{2R}$$

where R is the ratio of the greater to the smaller width.

$$R = W_W/W_N$$

For decreased rate—that is, flow from a narrow to a wider zone—the new rate is the reciprocal of this $1/G$.

This simple relationship is derivable from the following considerations.

If it is assumed that the rate of flow is the sum of two factors, the first of which we may call the capillarity factor and the second the accessibility factor, then in the case of the transition from a wide to a narrow strip, the capillary factor will be $1/R$ and the accessibility factor will be R.

Hence, the new rate will be:

$$V_2 = \left(\frac{1}{R} + R\right)$$

The original rate will be governed by conditions wherein both of these factors are unity. In other words,

$$W_W = W_N \text{ or } R = 1$$

Hence

$$V_1 = 1 + 1 = 2$$

Defining the gain, G, as $G = V_2/V_1$, we have:

$$G = \frac{1 + R^2}{2R} \qquad (2)$$

When $R \gg 1$, this reduces to:

$$G = \frac{R}{2} \qquad (3)$$

When $R_n = 10$,

$$\frac{1 + R^2}{2R} = 5.05 \text{ and } \frac{R}{2} = 5.00$$

Hence, for width ratios of 10 or greater, the use of Equation 3 instead of 2 leads to an error from this approximation of 1% or less.

The calculated and observed rates for the example plotted in Figure 3 are included in the diagram, and the data from which these rates are computed are shown in Table II.

Returning to the simple case represented by *A*, the question may be raised about the uniformity with which the strip is cut. Most chromatographers stress the fact that their sheets or strips of paper have been machine-cut. This is undoubtedly more convenient, but how does it affect the precision with which rates may be measured? This work indicates throughout that changes in dimensions are important. Measurements were, therefore, made on strips of the same dimensions of form *A*, some cut by machine and others with a pair of scissors. In the latter, no particular care was taken to preserve the maximum uniformity other than identical average dimensions. Typical results are shown in Tables III and IV.

Diffusion Coefficients

Machine-cut strip D = 203.5 mm.²/min.
Hand-cut strip D = 203.8 mm.²/min.

Table III. Effect of Uniformity of Strip

S. & S. 598 ST
Machine-cut 10-mm. rectangular strip
Eluant. Ethyl alcohol–water 84.6%
$n_D^{25} = 1.3630$
T = 25° C.

Time, Min.	Net Rise, Mm.	h^2
0	0.0	0
1	7.0	49
2	12.8	164
3	17.3	299
4	21.3	454
5	24.9	620
6	28.5	812
7	31.4	986
8	34.0	1156
9	36.6	1340
10	38.9	1513
11	41.3	1706
12	43.7	1910
13	45.9	2107
14	47.9	2294
15	50.2	2520
16	52.1	2714
17	54.1	2927
18	55.9	3125
19	57.9	3352
20	59.8	3576
21	61.6	3795
22	63.2	3994
23	64.9	4212
24	66.5	4422
25	67.8	4597
26	69.5	4830
27	70.8	5013

D = 203.5 mm.²/min.

This unusually close agreement indicates that there is no detectable difference. Indeed, from what is known about the influence of dimensional changes, one could predict with reasonable certainty that a strip cut on both vertical edges with a seamstress' pinking shears and thus having uniform serrations would yield rates identical with a plain rectangle. Actually, at each serration the rate would rise, only to fall at the next, and so on. There is a slight indication in the data of Tables III and IV that the average step-by-step variation in slope in the hand-cut strips is about 1.24 times greater than in the machine-cut strips, although the average slopes are identical to within 3 parts in 2000. This is pre-

Table IV. Effect of Uniformity of Strip

S. & S. 598 ST
Hand-cut 10-mm. rectangular strip
Eluant. Ethyl alcohol–water 84.6%
Temperature 25° C.
n_D^{25} = 1.3630

Time, Min.	Net Rise, Mm.	h^2
0	0.0	0
1	8.5	72
2	15.3	234
3	20.5	420
4	25.3	640
5	29.2	853
6	32.8	1076
7	36.0	1296
8	39.1	1529
9	41.9	1756
10	44.6	1989
11	46.7	2181
12	49.0	2401
13	51.4	2641
14	53.1	2820
15	55.1	3036
16	56.9	3238
17	58.5	3422
18	60.3	3636
19	61.8	3820
20	63.2	3994
21	64.5	4160
22	65.8	4330
23	67.8	4597
24	69.2	4789
25	71.0	5041
26	72.4	5242

D = 203.8 mm.²/min.

cisely what would be expected if hand cutting introduces random dimensional changes. Only a detailed least squares computation would evaluate this, but would contribute absolutely nothing to the main consideration; it makes no difference in the over-all rate.

The case represented by C, Figure 2, in which a rectangular strip terminates at the bottom in a pedestal, resolves itself into two special cases, both of which have been covered. If the pedestal is completely immersed in the liquid, its width has no influence on the rate of diffusion and it is indistinguishable from a simple rectangle like A. If only a portion of the pedestal is immersed, the state of affairs is identical with shape D and the augmented rates are as shown in Figure 3, depending only on the width ratio, R.

Table V. Effect of Shape of Strip

S. & S. 598 ST
Type J, Figure 2
Eluant. Ethyl alcohol–water 84.6%
Temperature 25° C.
n_D^{25} = 1.3630

Time, Min.	Net Rise, Mm.	h^2
0	0.0	0
1	0.6	4
2	9.8	96
3	16.3	266
4	21.4	458
5	25.9	671
6	29.7	882
7	33.2	1102
8	36.9	1362
9	39.8	1584
10	42.6	1815
11	45.2	2043
12	47.6	2266
13	50.0	2500
14	52.3	2735
15	54.5	2970
16	56.3	3170
17	58.3	3399
18	60.0	3600
19	61.7	3807
20	63.6	4045
21	65.2	4251
22	66.7	4449
23	68.3	4665
24	69.7	4858
25	71.1	5055

D = 206.3 mm.²/min. D = 237.3 mm.²/min.

The case represented by J, Figure 2, in which a rectangular strip of 10-mm. width gradually tapers to a 6-mm. width and then once more tapers back to the original width, behaves in a predictable manner. Typical data are given in Table V.

A group of three straight lines results from the square-law plot. When the liquid rises beyond the rectangle into the taper, it speeds up. Upon leaving the constriction, the rate decreases. The data yield a value for the slower rate of:

$$D = 206 \text{ mm.}^2/\text{min.}$$

and for the rate preceding it of:

$$D = 237 \text{ mm.}^2/\text{min.}$$

Gain G is therefore:

$$G_{\text{obsd.}} = 0.87$$

The calculated gain, according to Equation 2, is:

$$G_{\text{calcd.}} = 0.88$$

In another example for the same shape, J, the data may be examined in another way. The data of Table VI yield three straight lines, the respective values of D of which are:

$$D_1 = 209 \text{ mm.}^2/\text{min.}$$
$$D_2 = 226 \text{ mm.}^2/\text{min.}$$
$$D_3 = 194 \text{ mm.}^2/\text{min.}$$

As before, if the gain, as defined and calculated in Equation 2, is used to predict the slowest rate in terms of the rate immediately preceding it, then:

$$G_{\text{obsd.}} = 0.86$$
$$G_{\text{calcd.}} = 0.88$$

Table VI. Effect of Shape of Strip

S. & S. 598 ST
Type J, Figure 2
Eluant. Ethyl alcohol–water 84.6%
Temperature 25° C.
n_D^{25} = 1.3630

Time, Min.	Net Rise, Mm.	h^2
0	0.0	0
1	6.0	36
2	12.1	146
3	17.6	310
4	21.7	471
5	25.9	671
6	29.5	870
7	32.9	1082
8	35.8	1282
9	38.9	1513
10	41.7	1739
11	44.6	1989
12	47.4	2247
13	50.5	2550
14	52.5	2756
15	54.3	2948
16	56.1	3147
17	58.8	3457
18	60.8	3697
19	62.5	3906
20	64.2	4122
21	65.9	4343
22	67.2	4516
23	68.21	4651
24	70.1	4914
25	71.6	5127

D_1 = 209 mm.²/min.
D_2 = 226 mm.²/min.
D_3 = 194 mm.²/min.
Av. $D_{1,3}$ = 202 mm.²/min.

Furthermore, because the initial and final rates are too fast and too slow, respectively, as a result of the geometry, their mean value should be identical with that for a rectangular strip:

$$\frac{1}{2}(D_1 + D_3) = 202 \text{ mm.}^2 \text{ per minute in this example}$$

This is in good agreement with the values of D = 203.5 and 203.8, previously described for machine- and hand-cut rectangles of 10-mm. width.

Case E, in which a rectangular strip has two triangular notches

Table VII. Effect of Shape of Strip

S. & S. 598 ST
Type *E*, Figure 2
Eluant. Ethyl alcohol–water 84.6%
Temperature 25° C.
$n_D^{25} = 1.3630$

Time, Min.	Net Rise, Mm.	h^2
0	0.0	0
1	4.1	17
2	10.1	102
3	14.5	210
4	18.6	346
5	22.2	493
6	25.3	640
7	28.2	795
8	30.8	949
9	33.4	1116
10	36.0	1296
11	38.4	1475
12	40.4	1806
13	42.5	1806
14	44.8	2007
15	46.7	2181
16	49.0	2401
17	50.5	2550
18	52.8	2788
19	54.6	2970
20	56.5	3192
21	58.4	3411
22	60.2	3624
23	62.0	3844
24	63.7	4058
25	65.4	4277
26	67.3	4530
27	68.6	4706
28	70.2	4928
29	71.7	5141

$D_1 = 228$ mm.²/min.
$D_2 = 182$ mm.²/min.

Table VIII. Effect of Shape of Strip

S. & S. 598 ST
Type *G*, Figure 2
Eluant. Ethyl alcohol–water 84.6%
Temperature 25° C.
$n_D^{25} = 1.3630$

Time, Min.	Net Rise, Mm.	h^2
0	0.0	0
1	3.6	13
2	10.6	112
3	16.3	266
4	21.3	454
5	25.5	655
6	29.7	882
7	33.5	1122
8	36.9	1362
9	40.5	1640
10	43.5	1892
11	45.8	2098
12	49.2	2421
13	51.9	2694
14	54.3	2948
15	56.6	3204
16	59.1	3493
17	61.3	3758
18	63.4	4020
19	65.7	4316
20	67.7	4583
21	69.9	4886
22	71.9	5170

$D = 271$ mm.²/min.

cut into the edges, is also predictable, but, in this case, the special shape requires the gain formula to be modified to:

$$G = \frac{1 + R}{2R} \tag{3}$$

because the capillary factor is unity and only the accessibility factor changes. Two diffusion rates are observed in this example, the data for which are in Table VII:

$D_2 = 182$ mm.²/min.
$D_1 = 228$ mm.²/min.

According to Equation 3, the gain is calculated as:

$$G_{\text{calcd.}} = 1.250$$

The ratio of the above diffusion coefficients yields:

$$G_{\text{obsd.}} = 1.253$$

The mean of these two values is very close to the average value for a rectangular strip ($D = 204$):

$$\frac{1}{2}(D_1 + D_2) = 205$$

The remaining cases—*F*, *G*, *H*, and *I*—have not been evaluated in detail for two reasons. These patterns were chosen and measurements were made before the theory of their behavior had been worked out. The behavior of case *F* is very complex because the liquid must divide between two channels and reunite after leaving them. The theory has not been developed and has little utility because, experimentally, the turbulence at each transition extends over a good part of the range. Qualitatively, the results are satisfactory.

Table IX. Effect of Shape of Strip

S. & S. 598 ST
Triangular strip, apex down
Type *H*, Figure 2
Eluant. Ethyl alcohol–water 84.6%
Temperature 25° C.
$n_D^{25} = 1.3630$

Time, Min.	Net Rise, Mm.	h^2
0	0.0	0
1	3.9	15
2	9.5	90
3	14.1	199
4	17.8	316
5	21.2	449
6	24.2	586
7	27.1	734
8	29.5	870
9	31.9	1018
10	34.2	1170
11	36.4	1325
12	38.5	1482
13	40.5	1640
14	42.6	1815
15	44.3	1963
16	46.1	2125
17	47.8	2285
18	49.5	2450
19	51.0	2601
20	52.6	2767
21	54.0	2916
22	55.5	3080
23	56.8	3226
24	58.0	3364
25	59.4	3528
26	60.6	3672
27	61.8	3819
28	63.0	3969
29	64.3	4135
30	65.3	4264
31	66.4	4409
32	67.4	4543
33	69.7	4858
34	70.7	4999
35	71.7	5141
36	72.2	5213
37	72.5	5256

$D = 162$ mm.²/min.

The other cases add very little that is useful in comparison with the examples that have been discussed in detail.

For example, in *G* and *H* (Tables VIII and IX) the triangular strips yield diffusion rates of:

$D_1 = 271$ mm.²/min.
$D_2 = 162$ mm.²/min.

The considerable difference in rate for base down and apex down is evident. The median value:

$$\frac{1}{2}(D_1 + D_2) = 217$$

is somewhat larger than the average rate for a rectangular strip ($D = 204$).

In these tests, there is considerable uncertainty about depth of immersion and the results have qualitative interest only.

SUMMARY

The net result of this study of geometric factors is an understanding of the dynamics of the process from the purely physical

standpoint. The effect of abrupt discontinuities and the ability to predict them may be the key to speeding up chromatographic separations.

Indeed, the earlier technique of recording chromatograms during their development (*1*) owed its speed and resolution largely to the use of constricted paraffin channels on the paper. As shown there, detectable separations became apparent in 10 or 20 seconds and complete separations of some binary systems were achieved in a minute or less.

The present studies have shown how relative diffusion rates are predictably affected by shape factors. From both this information and the earlier practical application one can say that within the limits of flooding, geometric or shape factors can be applied to advantage in speeding up the chromatographic process.

It now remains to be shown how the diffusion rate in a given paper structure is influenced by its previous history and the physical properties of the eluting liquid. This is discussed in the following paper.

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RECEIVED August 30, 1950.

Kinetic Studies

THE present study is concerned with the previous history of the paper and the physical properties of liquids, from which it is possible to predict relative diffusion coefficients for a given system.

Previous work (*2*, *3*) has shown that all the data for the rate of rise of liquids can be fitted to an empirical square law well within the experimental error. This is of the form:

$$h^2 = Dt - b \qquad (1)$$

where h = height in millimeters
t = time in minutes
b = constant and equivalent to an h_0^2 term
D = constant for a given paper and liquid

A logical explanation of the phenomenon required that the rate of rise should be proportional to the surface tension of the liquid and inversely proportional to its viscosity. As accurate data became available, this was found to be the case. It was not possible to ignore the density of the liquid, and it became evident that the diffusion coefficient, D, was proportional to $\gamma/\eta d$.

Now, although Equation 1 represents the data with high precision, it leads to infinite height at infinite time, so that for the ascent of liquids in a vertical strip, it must break down for very large values of t. In all measurements here reported, the distance traversed was too small to make this limitation apparent, and this equation has been retained for its simplicity and precision (*2*).

The following equation eliminates this difficulty, if one assumes that the rate of rise is at all times proportional to the difference between the height and the maximum height attained when $t = \infty$. Hence:

$$\frac{dh}{dt} = \frac{k}{\eta}(h_m - h) \qquad (2)$$

where k equals a constant and it is assumed that the rate is also inversely proportional to the viscosity.

Now, when $dh/dt = 0$—i.e., at infinite time

$$h = h_m$$

and, as shown by Washburn (*6*), this should be equal to:

$$h_m = \frac{2\gamma \cos \theta}{rgd}$$

which is the maximum height to which a liquid rises in a capillary in infinite time.

Washburn also shows that, in horizontal capillaries, where the gravitational factor is absent, the rate of capillary flow is given by:

$$\frac{x^2}{t} = \frac{r\gamma \cos \theta}{2\eta}$$

This is precisely of the form of Equation 1 which is satisfactory for all data herein reported, and also indicates the respective roles of surface tension and viscosity.

Several conclusions may be stated at once.

In the Rutter disk technique (*2*, *3*, *5*) where flow is in a horizontal plane, the simple square law is adequate. Indeed, this has been found to hold, not only for the motion of solvent, but for the colored components carried by it (*4*).

For the rise in vertical strips, the square law will be adequate for moderate distances and elapsed times.

Returning to Equation 2, if we assume that

$$h_m = \frac{2\gamma \cos \theta}{rgh}$$

and represent this by a, then the integrated form will be:

$$\ln (a - h) = \frac{kt}{\eta} + \ln a$$

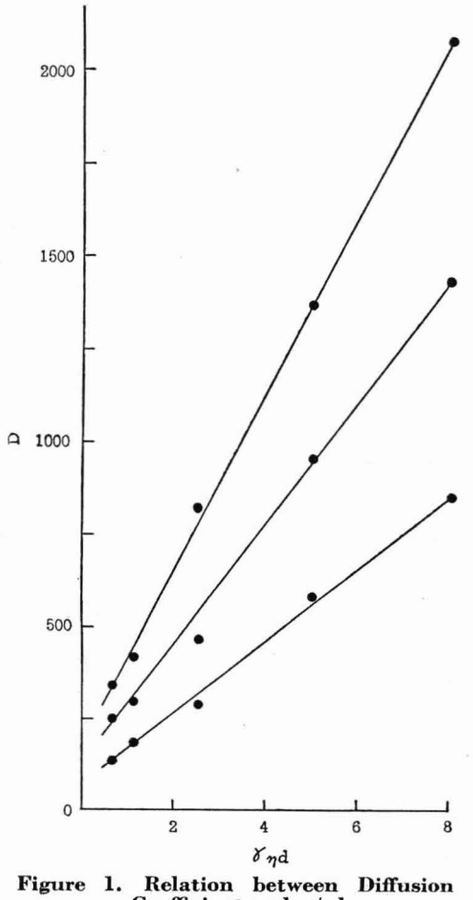

Figure 1. Relation between Diffusion Coefficient and $\gamma/\eta d$

The data fit this equation equally well, but it is extremely awkward for plotting or computing, in that suitable values for a must be assumed. Not only is this true mathematically for an equation of this sort, but from the nature of a it will be seen that several factors cannot be ascertained directly. As in most cases where surface tension is concerned, θ is assumed to be zero and the cos θ term is neglected. The real difficulty is presented by r, the capillary radius, and it is hard to imagine a more complex case than that presented by a mat of cellulose fibers.

That Equation 2 is of suitable form may be shown by assuming any arbitrary value for the maximum height, h_m, and plotting the data semilogarithmically. A straight line results with the intercept for $t = 0$ equal to h_m.

No data have been plotted in this form, for the simple reason that a much more direct and useful correlation may be obtained by relating the diffusion coefficient value, D, to γ/ηd for various liquids and papers.

A recent symposium, published in *Discussions of the Faraday Society* (*1*), contains a large number of papers devoted to the diffusion of liquids through porous media, few of which are directly applicable to these studies, but which confirm and support the general conclusions arrived at in this study.

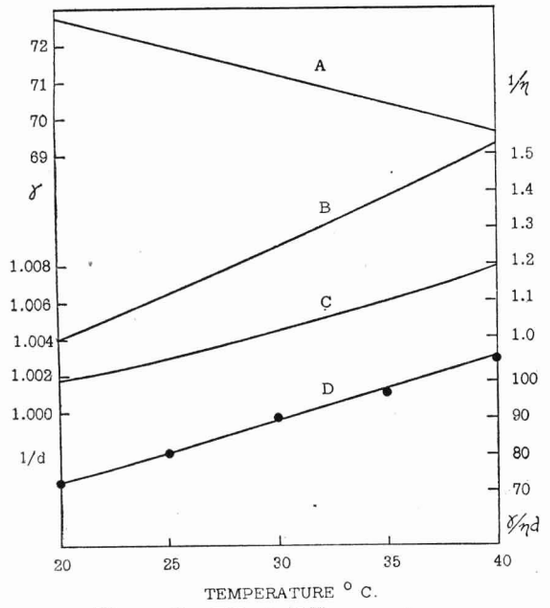

Figure 2. Effect of Temperature

A. **Surface tension of water**
B. **Reciprocal viscosity (fluidity) of water**
C. **Reciprocal density of water**
D. **γ/ηd of water**
● **γ/ηd of water, calculated from diffusion rate in paper**

A direct check on the assumption made above is afforded by the data shown in Figure 1, wherein the diffusion coefficients, D, for three different papers have been plotted against γ/ηd for five different liquids. The papers were Schleicher & Schüll, Nos. 601, 598 W, and 598 ST. From a great variety of papers, these three were selected primarily on the basis of speed of diffusion, 601 being very fast and 598 ST relatively slow, with 598 W intermediate. The liquids examined in this case were distilled water, methanol, ethyl alcohol, butyl alcohol, and amyl alcohol. In this and all other systems, the liquids were checked repeatedly in a thermostated Abbe refractometer. All measurements were made at 25° ± 0.02° C.

The surface tension, viscosity, and density values were taken from the International Critical Tables as noted in Table I. As usual, with information from this source, considerable cross plotting or computation from empirical equations was frequently necessary to get the information at the desired temperature. From these the values of γ/ηd were computed. The graphs shown in Figure 1 are the observed diffusion coefficients D as measured in the three papers. The upper or steepest line is for 601, the next for 598 W, and the lowest for 598 ST. Though not identified on the graph, the highest value for γ/ηd represents water and the smallest that for amyl alcohol.

Table I. Surface Tension, Viscosity, and Density Values

Substance	γ, 25° C.	η, Mp.[a], 25° C.	d 25° C.	γ/ηd
Water	71.97	8.949	0.9971	8.065
Methanol	22.17	5.531	0.7868	5.094
Ethyl alcohol	21.83	10.829	0.78506	2.568
1-Butanol	24.18	25.740	0.8064	1.165
Amyl alcohol	23.40	42.660	0.8090	0.678

[a] Mp. represents millipoise.

The lines drawn through the points can be represented in each case by an equation of the form:

$$D = a\gamma/\eta\text{d} + b$$

and they are, respectively:

For 601 $D = 238\gamma/\eta\text{d} + 167$
For 598 W $D = 163\gamma/\eta\text{d} + 115$
For 598 ST $D = 97.1\gamma/\eta\text{d} + 68$

Table II shows the observed and calculated values of D and the individual and average errors.

From these results, it may be seen that one can predict the diffusion rates through a given paper to about 3 to 6% on the basis of literature values of surface tension, viscosity, and density. This is very useful and convenient, but if detailed and exact measurements, particularly of surface tension, are made in the system at the time of measurement, much more accurate values can be obtained.

Of greater significance is the wide range over which this relationship holds, for the rate for water is many times greater than that for the higher alcohols. The rates for ethyl ether and benzene were predicted to be high from their γ/ηd values; indeed, ether is considerably higher than water, but in both cases very considerable difficulty was encountered in maintaining adequate vapor saturation, so that no very accurate measures of their diffusion coefficients were obtainable.

TEMPERATURE EFFECTS

In order to measure the influence of temperature on the diffusion process, many measurements were made at five different

Table II. Observed and Calculated Diffusion Coefficients

	γ/ηd	Dcalcd.	Dobsd.	Error %
	Paper 598 ST			
Water	8.065	851	851	0.00
Methanol	5.094	563	580	2.93
Ethyl alcohol	2.568	317	283	12.00
Butanol	1.165	181	181	0.00
Amyl alcohol	0.678	134	132	1.52
			Av.	3.3
	Paper 601			
Water	8.065	2086	2086	0.00
Methanol	5.094	1379	1370	0.66
Ethyl alcohol	2.568	778	824	5.58
Butanol	1.165	444	416	6.73
Amyl alcohol	0.678	328	328	3.53
			Av.	3.3
	Paper 598 W			
Water	8.065	1427	1427	0.00
Methanol	5.094	944	952	0.84
Ethyl alcohol	2.568	533	463	15.10
Butanol	1.165	305	293	4.10
Amyl alcohol	0.678	226	250	9.60
			Av.	5.9

temperatures. For each of these the thermostat was carefully set for 20°, 25°, 30°, 35°, and 40° C. At each of these temperatures, the diffusion of pure water was measured. Several other systems were measured, but the values for water are given here because there seems to be little uncertainty about the exact values of surface tension, viscosity, and density over this range of temperatures. The observed values are given in Table III and are plotted in Figure 2.

Table III. Effect of Temperature

$D_{obsd.}$, Mm.²/Min.	t, ° C.	$\gamma \pm 0.05$	η, M.P.	d	γ/ηd
696	20	72.75	10.050	0.99823	7.252
770	25	71.97	8.937	0.99707	8.077
840	30	71.18	8.007	0.99567	8.928
891	35	70.38	7.225	0.99406	9.799
963	40	69.56	6.560	0.99224	10.687

The manner of plotting the results in Figure 2 is thought to emphasize more clearly the contribution of each variable. The abscissas represent temperature in degrees centigrade and, beginning at the top, are the viscosity of water, next the reciprocal viscosity or fluidity, and next the reciprocal density. From these three, it is seen that, although surface tension decreases with increasing temperature, the reciprocals of viscosity and density increase. The net effect, γ/ηd, which is of special interest here, is seen to rise slightly with increasing temperature and very nearly linearly. The dots superimposed on this function are calculated from the observations of the rate of diffusion of water through the paper. The agreement is very good; indeed, as shown in Table IV, they may be computed to about 1%.

Table IV. Calculation of D for Water in 598 ST

From $D = 8.17\ \gamma/\eta$d + 100

t, ° C.	γ/ηd	$D_{calcd.}$	$D_{obsd.}$	% Error
20	7.252	692	696	0.6
25	8.077	768	770	0.3
30	8.928	830	840	1.2
35	9.799	901	891	1.1
40	10.687	973	963	1.0
				Av. 0.84

These results indicate that although the temperature coefficient is small, it can be predicted on the basis of the temperature variation of γ/ηd.

ETHYL ALCOHOL–WATER MIXTURES

An additional check on the utility of γ/ηd as a means of predicting diffusion rates in paper was obtained by measuring several ethyl alcohol–water mixtures over the entire range of composition. Table V gives the composition of the various mixtures and the calculated values of γ/ηd. These are plotted in Figure 3, in which the solid line represents γ/ηd as a function of composition and the solid dots are γ/ηd values recalculated from the measured values of D. The general form of the curve is followed faithfully, but the precision is inferior to those observed for pure water. The combined results of Figures 1, 2, and 3 leave no doubt that γ/ηd is a reliable index of the diffusion rates.

A few additional results indicate how the precision of these results may be greatly affected by minor factors.

When it was decided that a critical test of these results would be given by adding a surface tension depressant, some rates were measured on pure water saturated with caprylic alcohol. This is notoriously effective and is commonly used to suppress foaming in aqueous solutions.

Table V. Composition of Mixtures

Wt. Ethyl Alcohol, %	Surface Tension	Viscosity, Mp.	Density	γ/ηd
0	71.97	8.94	0.9971	8.065
10	47.5	13.2	0.98043	3.648
20	37.7	18.0	0.96639	2.158
30	32.4	22.0	0.96067	1.547
40	29.63	23.7	0.93148	1.340
50	27.90	23.6	0.90985	1.295
60	26.60	22.3	0.88699	1.344
70	25.40	20.2	0.86340	1.452
80	24.3	17.3	0.83911	1.666
90	23.4	14.2	0.81362	2.023
100	22.03	11.0	0.78506	2.549

The surface tension of the solution was carefully measured by the capillary rise method. Changes in viscosity and density were neglected, inasmuch as the solution is very dilute. A new value of γ/ηd was then computed to see if the decreased rate of diffusion could be predicted. The results were very disappointing and as much as 20% in error, although in the right direction. As a routine check on the surface tension some of the liquid from the diffusion chamber was remeasured after the diffusion run. Its surface tension had increased, and when this second value of γ was used to compute γ/ηd, the predicted rate was in excellent agreement with the observations. This was confirmed for three different papers and the results are shown in Table VI. The calculation in each case uses constants in the equation which were obtained from the rates for four alcohols and water (Figure 1).

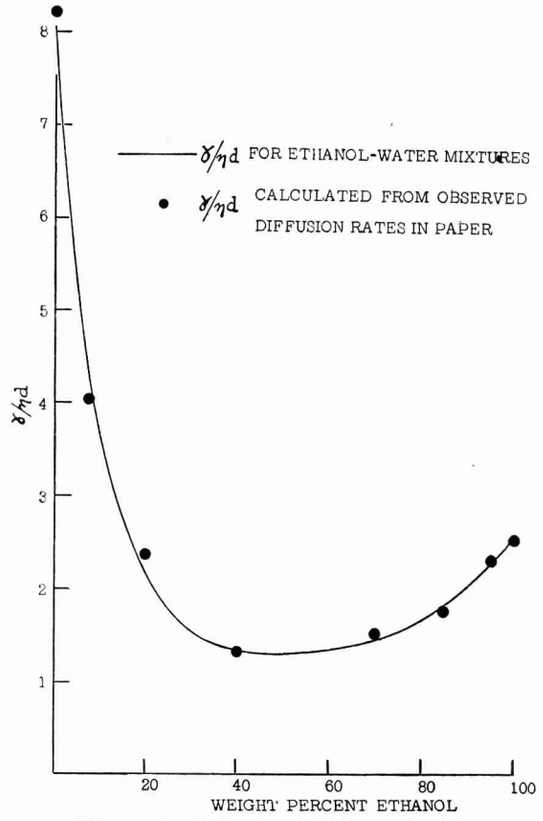

Figure 3. Calculated Values of γ/ηd

There is little doubt that, in this case, some caprylic alcohol was adsorbed in the chamber, or otherwise lost to the system, and the correct value of γ is that actually exhibited by the liquid as it enters the paper.

As all compilations of surface tension emphasize, there is considerable discrepancy among the values recorded in the litera-

ture. Furthermore, it is a property notoriously influenced by traces of impurities. Because there is ample evidence in this work that $\gamma/\eta d$ as computed from literature values is a fairly precise means of predicting diffusion rates, this is itself of great potential use to the chromatographer. The latter experiments also indicate that considerable precision can be obtained if one measures the surface tension of the liquid after it has been in contact with the system.

REPEATED DIFFUSION IN SAME PAPER

An alternative approach to checking surface active substances is to measure the diffusion rate and then dry the strip in situ and repeat the diffusion measurements with more of the same liquid. Within limits, this can be repeated indefinitely, although the physical texture of the paper may gradually change if this is done too often. When this was tried with water, followed by careful, warm-air drying, the rate increased and after several runs started to decrease very slightly. The increase was undoubtedly due to the washing out of residual impurities by the first, and less by the second runs. A small decrease could arise from new impurities picked up, but much more likely from structural changes in the fibers or their orientation. Inasmuch as texture and physical structure have an enormous influence, it is probably too much to expect to secure a final equilibrium condition. These results are entirely in accord with some limited observations of Le Strange in this laboratory. In measurements on the electrolytic conductance of paper strips through which solutions or pure liquids were diffusing, he never failed to observe a strong wave of increased conductance as the solvent crossed the electrodes. This always subsided as more liquid came along and, therefore, indicated some conducting material in the advancing liquid front. Obviously, this technique would respond only to ionic species, whereas, in general, any solute might well alter the surface tension.

Table VI

Paper	$\gamma/\eta d$, Water	$\gamma/\eta d^a$, Water and Alcohol	$D_{calcd.}$, Mm.²/Min.	$D_{obsd.}$, Mm.²/Min.	% Error
601	8.07	6.02	1600	1643	2.62
598 W	8.07	3.82	737	734	0.41
598 ST	8.07	6.02	652	657	0.76

a Remeasured after contact with system.

Pretreatment of paper, such as washing and drying, has been recommended in the literature of chromatography. In some instances the presence of ultramicro traces of substances, such as copper, has profound effects, but usually for chemical reasons.

INFLUENCE OF VAPOR SATURATION

In all chromatographic work, it is essential to keep the paper in an environment saturated with the vapor of the eluting liquid. In this work, vapor saturation was very carefully controlled; indeed, this was indispensable for securing good checks and reproducible results. However, it may be shown that the attainment of equilibrium conditions requires considerable time. Table VII shows the progressive increase in diffusion rate as a function of time of saturation. The rate becomes constant after 30 minutes, but the paper of itself continues to absorb vapor for a much longer time. Two series of measurements, the details of which are not repeated here, confirm this conclusion.

In one case a strip of paper was suspended from one arm of a chainomatic balance in a nearly vapor-tight tube saturated with water vapor. The increase of weight was followed as a function of time and, although rapid in the beginning, several days were required before equilibrium was attained. In another instance, a strip of paper was clamped between two electrodes in a completely vapor-tight enclosure. The resistance of the strip was about 1000 megohms initially and reached an equilibrium value of about 30 megohms in several days.

The results by the two methods were very similar and indicate that, whereas a steady state for the diffusion of liquid is attained after about 30 minutes of vapor presaturation, this actually represents about half the total amount of water vapor which the paper can absorb from the saturated atmosphere.

This affords strong evidence that the accommodation of moisture in the fiber mass is a complex matter and bears no simple relationship to the subsequent diffusion of the liquid phase through it. Many more experiments would be required to understand this phenomenon.

Table VII. Influence of Saturation Time on Diffusion

Time of Saturation, Min.	D, Mm.²/Min., Paper 601
10	468
20	470
40	485
60	485

The effects of vapor saturation, previous preparation of the paper, and surface active impurities might well seem to present insuperable difficulties in this work. Actually, one must recognize the compromise which the chromatographer must make. The properties of filter paper are not "constants of nature" but characteristics of a complex system. In practical work, conditions are so chosen that there is a constant intercomparison between solvent and solute motion, and the various factors may be expected to compensate somewhat.

Enough has been established on the kinetics of solvent motion to enable one to evaluate different papers. However, as this is but a part of the chromatographic technique, the automatic recording of solvent and solute motion should furnish one with all the desired information about a given paper.

SUMMARY

Semiautomatic methods for recording the diffusion rates of liquids through paper yield values which follow a square law with high precision. The diffusion coefficients so obtained are a simple function of $\gamma/\eta d$ of the liquid.

This is strikingly illustrated for the binary system, ethyl alcohol–water, which obeys the relationship over the entire composition range.

The positive temperature coefficient is completely explained by the temperature coefficient of the quantity $\gamma/\eta d$.

As it is known that both solutes and solvent follow the square law, a knowledge of the laws governing solvent behavior is an important, though incomplete criterion, of chromatographic separation.

The complete information will be obtained when the motion of both solvent and solute are followed simultaneously, and automatic methods are in the process of development for this purpose. On the basis of such methods, one may hope to attain a rapid and distinctive criterion of papers suited for any chromatographic separation.

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Multiple-Paper Chromatogram

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A method was required by which mixtures of organic acids or alkaloids in semimicroquantities could be resolved into their pure components to yield sufficient amounts for the preparation of derivatives for identification. A new apparatus, the "Chromatopack," consists of a pack of Whatman No. 1 filter paper strips compressed between two stainless steel plates. The zones obtained from mixtures of pure compounds were separated and yielded up to 10 mg. of each constituent. The method lends itself to easy detection of zone positions with resolution equal to that obtained with single sheets of paper but producing semimicro quantities of pure materials. This extends the usefulness of paper chromatography from a submicroqualitative tool to a semimicropreparative tool.

THE usefulness of the papergram technique in the resolution of mixtures of many kinds of materials has been established. The original technique of Consden *et al.* (*1*) has been modified in order to handle smaller and smaller amounts of materials (*6*). However, for preparative work and for identification experiments, where known materials are not available, these procedures are not satisfactory and modifications such as the "Chromatopile" (*3*, *4*) have been adopted. In this laboratory the Chromatopile has proved somewhat inconvenient because of the formation of zones shaped like "inverted cones," which made it difficult to separate the fractions, and the operation of detecting the zones by removing many sheets from the pile was relatively tedious. As a result, a new technique has been developed which requires the simplest apparatus and lends itself to easy detection of the zones and to their ultimate isolation and elution. The setup has been termed a "Chromatopack."

A photograph of the Chromatopack is shown in Figure 1.

It consists of a pack of long strips of Whatman No. 1 paper (18 × 2 inches or wider) clamped between two stainless steel plates. The strips are cut from the usual 18.25 × 22.5 inch sheets by means of a paper cutter. For use, the sample is placed on a line 2.0 cm. from one end of each of 100 or more strips, using about 0.01 ml. of solution per centimeter of width. A No. 26 hypodermic needle and a 0.5-ml. syringe with the plunger removed are used to distribute the same solution. Ten blank strips are placed on each side of the pack of sample strips. The entire pack is carefully aligned and placed between the stainless steel plates, so that the end on which the sample was deposited is about 5 mm. from the end of the plates. After tightening the nuts to compress the sheets, the entire assembly is placed upright in a 12 by 24 inch (30 × 60 cm.) glass cylinder with the sample end resting in a stainless steel tray. The solvent, either the organic phase of the equilibrated two-phase solvent or a one-phase solvent containing water, is carefully added to the tray and the chromatogram is developed until the solvent front has moved to just below the top of the pack. At this time, the pack is removed, the plates are loosened, and the filter paper pack is withdrawn. A sheet from each side and from the center of the pack is removed, dried, and sprayed for detection of the bands. The pack is cut and the different zones are isolated. These fractions are eluted and subjected to qualitative analysis. No quantitative analyses have been carried out to date, but several investigations are in progress.

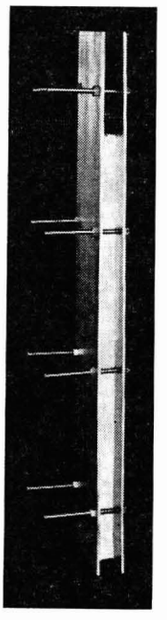

Figure 1. Filter Paper Chromatopack after Removal from Solvent Chamber

EXPERIMENTAL

The technique was tested using dyes, nonvolatile organic acids, and tobacco alkaloids. Chromatopacks consisting of 200 strips (18 × 2 inches), each containing the solute from 0.05 ml. of sample solution, were assembled, placed in the glass cylinder, and developed until the solvent front had moved in excess of 28 cm. At this time the pack was disassembled and the test sheets were removed, dried, and sprayed for detection of the zones.

The individual components of the mixtures, their concentration, range of spot movement, and their R_F values, as well as the solvent and spray materials, are summarized in Table I.

DISCUSSION

The procedure of placing the sample on the separate sheets is somewhat tedious, but it can be eased by placing the sample on the large sheets of paper (18.25 × 22.5 inches) prior to cutting the strips. If precut strips are available, a microburet having a mechanical delivery attachment can be used. The relative inefficiency of this step is more than overcome by the ease of assembly, detection of zone position, and isolation of the pure components which have been resolved from the mixture.

In the preliminary experiments with the organic acids,

Table I. Summary of Experimental Results Obtained Using Chromatopack

Type of Mixture	Components of Mixtures	Concentration *Mg./ml.*	Range of Spots *Cm.*	Solvent Front *Cm.*	R_F	Solvent Composition *Parts or ml.*	Spray Mixture
Dyes	Fuchsin G	5	0.3–1.8	28	0.04	1-Butanol, 40	None needed
	Methylene blue	5	10.6–12.4		0.41	Abs. ethyl alcohol, 10	
	Crystal violet	5	22.9–28.0		0.91	Water, 50	
Organic acids	Tartaric	5	11.0–14.5	33.1	0.40	5 *M* formic acid, 50	Bromophenol blue
	Malic	15	16.9–18.7		0.53	1-Pentanol, 50	
	Succinic	15	20.9–24.3		0.68	Addnl. abs. ethyl alcohol to form one phase	
Tobacco alkaloids	Nornicotine	5	0.3–4.0	34.0	0.06	1-Butanol, 85	Iodine
	Nicotine	5	7.5–19.2		0.36	Benzene, 5	
	Nicotyrine	5	21.5–31.1		0.80	Buffer[a], 30	

[a] Mixture of 9.5 ml. of 0.2 *M* acetic acid, and 90.5 ml. of 0.2 *M* sodium acetate; pH, 5.6 (*5*)

the organic phase of the two-phase system of Lugg and Overell (*2*) contained insufficient formic acid to suppress the ionization of the organic acids and resulted in tailing and poor resolution. Addition of enough ethyl alcohol to cause the formation of a single-phase system produced the results shown in Table I.

ACKNOWLEDGMENT

The author expresses his appreciation for the help given by Margaret L. Buch in the experimental development of the Chromatopack technique.

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Paper Chromatography of Organic Acids

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The study of the paper chromatography of organic acids was undertaken to provide a simple method for the rapid identification of organic acids in sugar-beet processing liquors. The use of several developing solvent mixtures has been investigated. The R_F values for 18 organic acids were measured. The effect on R_F values of hydration of paper, water content of solvents, temperature of development, and presence of inorganic acids was studied. In many cases an inversion of R_F values was obtained by altering the composition of the developing solvent. The method is suitable for detection of many organic acids or their salts in plant extracts or other biological media. The presence of acid impurities or their production as reaction products may be detected.

AN INVESTIGATION under way in this laboratory on changes in composition of sugar-beet processing liquors required a technique suitable for rapid and accurate identification of organic acids. The detection and identification of amino acids and other compounds by paper chromatography (*3–5, 10, 11*) suggested its application. During this work, methods were published for paper chromatography of acids in the form of derivatives (*2, 6*) or labeled with radioisotopes (*1*). The authors have studied the behavior of several nonvolatile acids in various solvent mixtures containing formic or acetic acid (*9*). The method discussed here is similar to that of Lugg and Overell (*8, 9*) but offers some advantages in identification. It has been used to identify some of the acids in sugar-beet processing liquors and to determine the efficacy of fractionation procedures.

METHOD

The paper sheets, 40 × 57 cm., Whatman No. 1, were developed by an ascending technique similar to that described by Williams and Kirby (*12*). Glass jars, 10^1/$_8$ × 18 inches, with glass covers were used to contain the solvent and paper. The sheets were clipped to a hexagonal rack made from 5-mm. glass rod. Only one solvent phase was present in the developing tank. The paper sheets were not humidified, nor was the temperature of the developing bath controlled; however, the laboratory temperature averaged about 25° ± 3° C. The papers were spotted with 0.003 to 0.01 ml. of solution 3 cm. from the bottom and 2 cm. apart. The standard acid solutions were 0.075 *N*. Development time was usually 16 hours, but periods as short as 2 hours were also satisfactory.

Following development, the papers were removed from the tank and either dried overnight in a hood or for 45 minutes at 100° C. in a circulating air oven. Better definition of spots was usually obtained on papers dried at room temperature; some acids are volatile or subject to decomposition at higher temperatures. Occasionally it was found advisable to hydrate the paper by exposure to water vapor before drying was finished, to remove traces of some acidic substances that might be present or formed on heating the paper and solvent. The position of the acids on the chromatogram was shown by spraying the dried paper with either bromocresol green or bromophenol blue, 400 mg. per liter in 95% alcohol. The indicator solutions were made slightly alkaline with sodium hydroxide. The acid spots were yellow against a blue or purple background. They were circled and the centers marked. R_F values were calculated for the individual spots.

Many of the solvents contained alcohols, and some esterification with formic or acetic acid took place. The small amount of esters present did not appreciably affect the R_F values, but the reduction in acid concentration caused considerable tailing. This was not serious during a period of a week or two, and if the solvent was not replaced in this time, a portion was titrated and make-up acid added. Every month the solvent should be replaced by fresh solution. The solutions were made up in stock bottles and the formic or acetic acid was added when the solvent was placed in the jar. Solvents should be checked for the presence of nonvolatile acids and purified, if necessary, before use.

EFFECT OF CONDITIONS OF EXPERIMENT

Effect of Hydration of Paper, Temperature of Development, and Water Concentration. A preliminary study was made of the effects of hydration of the paper, of changes in water concentration in the phenol-water–formic acid system, and of temperature during development in solvents F and G. These results are shown in Table I. In general, humidification of the paper has an influence similar to increasing the water concentration of the developer—that is, the R_F values of acids such as citric and aconitic are increased, while those that are high generally remain practically unchanged. In most cases temperature changes have only a slight influence. Because of the slight effect of temperature change and humidification, the chromatographic papers were not humidified and development was at room temperature.

Acid Concentration. Several acids were developed with 75% phenol and 25% water containing 0.2, 1, and 2% acetic acid.

Table I. Some Factors Affecting R_F Value ($R_F \times 100$)

Acid	Hydrated Solvent G[a] 25° C.	Hydrated Solvent G[a] 25° C.	Hydrated Solvent G[a] 35° C.	Nonhydrated 80%[c]	Nonhydrated 70%[d]	Solvent F[b] 25° C.	Solvent F[b] 35° C.
Aconitic	45	37	42	34	44	71	65
Adipic	85	82	82	82	88	82	80
Citric	32	25	27	19	30	35	35
Fumaric	61	60	60	56	61	78	76
Glutaric	78	76	78	79	81	79	77
Glycolic	56	58	60	59	60	54	54
Lactic	70	72	75	76	76	64	67
Maleic	50	50	58	55	59	38	37
Malic	42	43	41	36	44	46	46
Malonic	48	47	49	46	50	56	51
Oxalic	21	21	21	15	25	11	7
Methylene bis-*N*-pyrrolidone carboxylic	87	85	86	87	86	64	66
Pyrrolidone carboxylic	86	86	87	85	86	59	63
Succinic	72	65	68	61	71	72	72
Syringic	94	95	90	91	90	87	84
Tricarballylic	59	49	50	42	56	67	65
Trihydroxy glutaric	25	21	24	14	26	16	24

[a] Solvent G = phenol, 3 grams; water, 1 ml.; 90% formic acid, 1%.
[b] Solvent F = isopropyl alcohol, 1 ml.; *tert*-butyl alcohol, 1 ml.; water, 1 ml.; benzyl alcohol, 3 ml.; 90% formic acid, 2%.
[c] Phenol, 4 grams; water, 1 ml.; 90% formic acid, 1%.
[d] Phenol, 7 grams; water, 3 ml.; 90% formic acid, 1%.

Considerably less tailing and slightly higher R_F values were obtained with 1 or 2% acetic acid. Later experiments using formic in place of acetic acid to repress ionization yielded still better chromatograms. The authors have used it exclusively, but not at the high concentrations of 0.85 to 4.3 M used by Lugg and Overell (*8*). They allowed esterification equilibrium to be reached and used the organic phase for development.

Effect of Inorganic Acids. The interference by inorganic acids is dependent on their concentration. R_F values for hydrochloric, sulfuric, nitric, and phosphoric acids at 0.05 and 0.1 N were determined in solvents B and D (Table II). The inorganic acids moved about twice as far at 0.1 N as at 0.05 N. Phosphoric acid moved farther than the other acids, but in no case was the R_F greater than 0.1. Inorganic acids tend to complicate chromatograms of organic acids, because in low concentrations they obscure those with low R_F values and tend to increase the R_F of some of the others. In high concentrations inorganic acids may move a distance nearly equal to the solvent front, thereby ruining the chromatogram.

Table II. $R_F \times 100$ for Organic Acids

Acids	Solvents A	B	C	D	E	F	G
Aconitic	79	69	76	65	63	69	36
Adipic	84	88	92	83	80	86	86
Citric[a]	33	34	28	24	23	35	26
Fumaric	84	85	89	75	74	78	63
Glutaric	80	85	89	80	77	78	78
Glycolic[a]	60	57	62	48	48	54	59
Lactic[a]	75	76	83	65	61	64	72
Levulinic						85	91
Malic[a]	51	41	46	35	36	44	42
Malonic	64	51	70	45	43	53	48
Oxalic[a]	6	5	9	8	4	10	18
Pyrrolidone carboxylic[a]	45	57	69	52	56	60	84
Methylene bis-*N*-PCA	59	78	87	52	58	64	86
Succinic[a]	75	76	81	69	66	72	66
Syringic		88	95	86	85	86	95
Tartaric	15	17	13	13	12	24	19
Tricarballylic	71	65	66	60	59	64	52
Unknown[a]						34	49

Composition of developing solvents:
A = iso-octane, 4 ml.; 95% ethyl alcohol, 4 ml.; acetone, 1 ml.; 90% formic acid, 1%.
B = chloroform, 2 ml.; 95% ethyl alcohol, 1 ml.; 90% formic acid, 2%.
C = chloroform, 1 ml.; 95% ethyl alcohol, 1 ml.; 90% formic acid, 1%.
D = *n*-butyl alcohol, 5 ml.; benzyl alcohol, 5 ml.; water, 1 ml.; 90% formic acid, 1%.
E = *tert*-butyl alcohol, 5 ml.; benzyl alcohol, 15 ml.; water, 2 ml.; 90% formic acid, 1%.
F = isopropyl alcohol, 1 ml.; *tert*-butyl alcohol, 1 ml.; benzyl alcohol, 3 ml.; water, 1 ml., 90% formic acid, 2%.
G = phenol, 3 grams; water, 1 ml.; 90% formic acid, 1%.

[a] Acids found in sugar-beet processing liquors.

Effect of Various Solvents. A good developing solvent for chromatography of organic acids should give a wide range of relative movement for the acids being studied, contain some water or possibly formamide, be stable over a reasonable period, leave only neutral or slightly acidic substances on the paper if the spots are to be detected with an acid-base indicator, and contain no colored residue that will obscure the spots.

Several of the solvents tried may be classed as failures or partial failures on this basis. A list of these should be of interest in preventing duplication of effort.

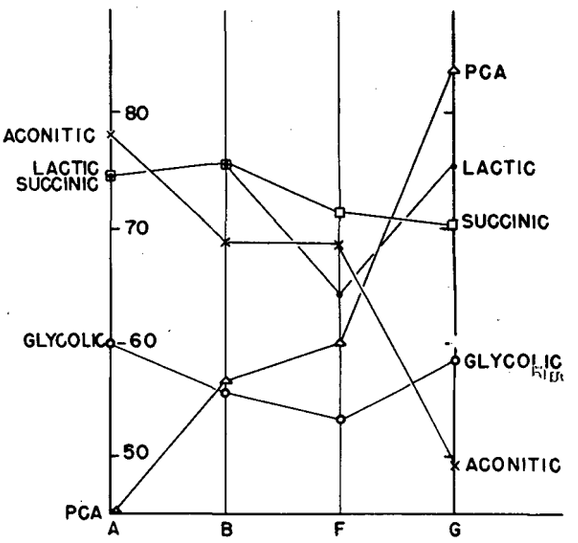

Figure 1. Inversion of R_F Values of Certain Organic Acids in Various Solvents

A. Iso-octane, alcohol, acetone
B. Chloroform, alcohol
F. Benzyl, isopropyl, *tert*-butyl alcohols
G. Phenol

Solvents with a high proportion of low-molecular-weight ketones or alcohols with water give R_F values over a narrow range near the solvent boundary. Acids developed with equal parts of isopropyl alcohol, *tert*-butyl alcohol, and water have R_F values of 80 or higher. Acetone, water, and acetic acid in the ratio of 13:5:3 yield similar chromatograms. A mixture of benzyl and *tert*-butyl alcohols without water gives only slight movement. The addition of water is necessary to produce sufficient movement for the separation to be useful. In fact, the addition of water to all developers seems to be necessary, although preliminary experiments indicate that the water might be replaced with formamide in some solvents. Acetone or alcohol must be added to iso-octane (2,2,4-trimethylpentane) or chloroform to permit the solution of a small amount of water before they can be used to separate acids in a mixture.

A solution of freshly distilled furfuryl alcohol and water, 8 to 2, forms very desirable chromatograms, but sufficient decomposition occurs in a day or two to leave a nonvolatile colored residue on the paper. Solutions of dioxane, acetone, and water, and of benzene, *tert*-butyl alcohol, acetone, and water, formed or contained nonvolatile acidic fractions that obscured the position of the test acids on the chromatogram. Solutions of carbon tetrachloride, water, and acetic acid, of xylene, water, and acetic acid, or of dioxane and toluene did not give sufficient movement of the acids. Collidine-lutidine-water mixtures did not adequately separate the acids. Similarly, *n*-propyl alcohol, ammonia, and water, recommended by Hanes and Isherwood (*7*), did not separate the acids with which the authors were concerned. The latter solution should be of value in identifying volatile acids, as the acids are present as the ammonium salts. Their position is indicated by spraying with ninhydrin. A mixture of phenol and

water forms a band of acid extending about one fourth the distance to the solvent front. In spite of this defect, a phenol-water solution is one of the best developing solvents. To eliminate obscuring of acids with R_F values less than 0.25, the initial spots may be made about 10 cm. from the bottom of the paper.

Table II gives the composition of several suitable solvent mixtures and the R_F values for 18 acids in these mixtures. As shown in the table, the presence of several of these acids in sugar-beet processing liquors has been confirmed. The advantages of developing separate sheets with two or more solvents are apparent in Figure 1, where the movements of five acids are compared in four solvents. Lactic and succinic acids vary by not more than five units in any solvent. In separate runs the difference may be much less. With solvent F the succinic acid spot will be consistently higher than that of lactic acid. With solvent G the positions are inverted. This inversion of R_F is noticed with several other acids investigated. Two-dimensional chromatography first with solvent F and then G does not resolve lactic and succinic acids as satisfactorily as separate one-dimensional chromatograms.

When identifying unknown acids it is necessary to compare the unknown with a known acid on the same paper. Day-to-day variations of R_F values may be as much as three or more points from the mean, but there is only a slight change in the R_F for the same acid in different spots on a single sheet. Unknown materials should be run in two or more solvent mixtures.

APPLICATION TO PLANT MATERIALS

The following general procedure is recommended in studies of acids present in plant extracts. The presence of acids in moderate amounts, 0.5% in dry solids, may be demonstrated by removal of cations with an ion exchanger and chromatographing the solution. To identify acids in lower concentrations, it is sufficient in many cases to adsorb the acids on an anion exchange resin, elute with ammonium hydroxide, remove ammonia on a cation exchanger, concentrate, and chromatograph the solution. In case one acid is present in a much greater amount than the others, it may be necessary to remove as much as possible of that constituent before chromatographing to prevent masking of other acids. The importance of checking results in more than one solvent cannot be overemphasized.

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Improved Techniques in Paper Chromatography of Carbohydrates

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To provide a basis for the extension of paper chromatography to the separation of disaccharides and oligosaccharides, a study was made of the interrelationships among the degree of resolution of the sugars, their R_F values, and solvent composition. In addition, a multiple development technique was worked out to improve the resolution of sugar mixtures while retaining all the components on the paper sheet. A self-supporting glass spiral for holding the paper permitted use of sheets of various widths when ascending movement of the solvent was employed, while for descending movement a glass support enabled use of lengths of paper greater than the height of the container. For detection of reducing sugars on the chromatograms, an alkaline 3,5-dinitrosalicylate reagent proved advantageous. Included in the study were six common monosaccharides, six reducing disaccharides, and a mixture of glucose, maltose, and oligosaccharides obtained by the action of malt α-amylase on amylose and presumably constituting the homologous series up to a degree of polymerization of at least ten glucose units. The relationships established provide a basis for selecting optimum conditions for the separation of a wide variety of reducing sugars. The techniques and apparatus described should prove useful in the application of paper chromatography to the analysis of other types of compounds.

THE use of paper chromatography for the separation of carbohydrates has dealt mainly with monosaccharides. In extending the method to reducing disaccharides and oligosaccharides, it was found necessary to study a number of new solvent combinations and to devise a new multiple development technique in order to obtain adequate separation of most of the sugars involved. The search for suitable solvents was on an empirical basis at first because insufficient information was available (*10*, *13*) on the interrelationships of the solvent composition, sugar structure, and degree of resolution. The present studies of such relationships provide a more logical basis for selecting the solvents most likely to give satisfactory separation of a wide variety of mono-, di-, and oligosaccharides. The multiple development technique was chosen as the best method that would improve separations yet maintain all sugar spots on the paper. Because both descending (Partridge, *13*) and ascending (Williams and Kirby, *16*) movement of the solvent have been used extensively, both techniques were included in this work.

Table I. Chromatographic Behavior of Simple Sugars with Various Developing Solutions, Single Development

	Solvent Composition and Ratio by Volume Before Separation							
	Ethyl Acetate–Acetic Acid–Water (*8*) 3:1:3	1-Butanol–Collidine–Water 3:1:1.5	1-Butanol–Collidine–Water 1:1:1	Fusel Oil–Acetic Acid–Water 4:1:1	Fusel Oil–Pyridine–Water 3:1:1.5	Ethyl Acetate–Pyridine–Water (*8*) 5:2:5	Fusel Oil–Pyridine–Water 3:2:1.5	1-Butanol–Pyridine–Water 3:1:1.5
Solvent layer, % total by volume	51	85	76	100	84	54	86	97
Running time, hours	8	19	19	22	18	10	15	18
R_F values × 100								
Fructose	6	8	9	12	11	15	27	28
Glucose	4	5	7	8	8	11	21	23
Maltose	1	1	2	2	2	4	9	12
Cellobiose	1	1	1	2	2	3	8	12
Ratio $\frac{R_F \text{ maltose}}{R_F \text{ glucose}} \times 100$	14	22	25	27	27	35	45	54
Separation of centers of spots (R_F glucose–R_F maltose) × 100	3	4	5	6	6	7	12	11

	Solvent Composition and Ratio by Volume Before Separation							
	1-Butanol–Pyridine–Water 3:1.3:1.5	1-Butanol–Pyridine–Water (*4*) 3:2:1.5	1-Butanol–Pyridine–Water 3:2:1.5	Collidine–Ethyl Alcohol–Water 2:1.5:1	Fusel Oil–Pyridine–Water[a] 7:7:6	Fusel Oil–Pyridine–Water 1:1:1	1-Butanol–Pyridine–Water 1:1:1	Collidine–Pyridine–Water 2:1:1
Solvent layer, % total by volume	100	98	100	100	100	100	100	100
Running time, hours	20	17	19	16	18	22	18	16
R_F values × 100								
Fructose	32	42	41	37	39	40	56	59
Glucose	26	37	37	32	36	37	53	57
Maltose	16	24	24	21	27	31	45	50
Cellobiose	15	22	22	20	25	29	43	49
Ratio $\frac{R_F \text{ maltose}}{R_F \text{ glucose}} \times 100$	58	64	65	67	76	83	84	88
Separation of centers of spots (R_F glucose–R_F maltose) × 100	11	13	13	11	9	6	9	7

[a] Solution similar to that of Edman (*7*).

MATERIALS AND REAGENTS

Whatman's No. 1 filter paper was used throughout these studies.

The solvents, which were of commercial origin, were distilled before use. The crude fusel oil, obtained from cereal fermentation, was dried over potassium carbonate and the fraction distilling between 121° and 129° C. was used. This fraction, most of which distilled between 126° and 129°, is considered to consist of amyl alcohols (isobutyl carbinol and *sec*-butyl carbinol), probably with a small amount of isobutyl alcohol. (Some variation in the composition of this fraction can be expected, depending on the source of the fusel oil. No change in the R_F values of glucose and maltose occurred, however, when a second sample was used which gave an incompletely miscible 1:1:1 fusel oil–pyridine–water mixture.) The collidine was the symmetrical (*γ*) type. Solvent-water mixtures which were incompletely miscible were shaken mechanically and the organic phase was separated for use.

The sugars used in these studies were commercial products or laboratory preparations of high purity. Mixture A in Table II was obtained by the hydrolysis of potato amylose with malt *α*-amylase (*6*). Although the component oligosaccharides have not yet been isolated and identified, it seems highly probable that the mixture is the homologous series of *α*-1,4-linked polymers of glucose.

The reagent for detecting sugar spots is a modification of Sumner's dinitrosalicylate reagent for quantitative determination of reducing sugars (*14*). It is an aqueous solution containing 0.5% of 3,5-dinitrosalicylic acid and 4% of sodium hydroxide. This reagent offers the advantages of being a stable solution, giving a permanent record of the positions of reducing sugars as distinct brown spots on a pale yellow background, having a good degree of specificity for reducing sugars, and being relatively unaffected by ionic impurities and contact with metals. The dextrose in 1 μl. of 0.1% solution could be detected with this reagent on a developed chromatogram. Where higher sensitivity is required, other reagent combinations, such as those of Hough (*9*) and of Trevelyan *et al.* (*15*), may be used, sometimes with some sacrifice of convenience or of freedom from interference by substances other than reducing sugars. For specific classes of sugars, such as pentoses or ketoses, and for nonreducing sugars a number of useful reagents have been described in the literature [see, for example, the review by Clegg (*5*), also (*9*) and (*15*)].

APPARATUS

For preliminary runs by the ascending technique (*16*) a glass-stoppered 1-liter graduated cylinder was used with about 20 ml. of developing solution. The paper (8 × 39 cm.) was held in cylindrical shape by fastening with thread, instead of with staples or adhesives, to avoid possible effects of corrosion or of solubility of adhesive materials in contact with the solvents.

For large sheets of paper in the ascending method, a glass jar 28 cm. in diameter and 48 cm. high was closed by a glass pie plate, with the flange ground to fit tightly, and a cover of thin sheet rubber. A cylinder of heavy corrugated cardboard shielded the apparatus from drafts and radiation. The filter paper was held in a spiral similar to that described by Ma and Fontaine (*11*) but made of 3-mm. glass rod with the spirals in fixed positions. The outer turn of the bottom spiral was bent at three points to provide supporting U-shaped legs. Such a spiral, either of glass or stainless steel, is particularly effective in preventing the collapse of even a very large sheet of wet filter paper.

For descending chromatography the same kind of jar was used as for the ascending method. A glass scaffold (Figure 1, *a*) supported a glass boat, 2.5 × 2.5 × 20 cm., made according to the method of Atkinson (*1*) but with the addition of small bumps on the outside to prevent it from rotating. The ends of the papers were secured in the boat by a thick glass rod with sealed-on handle. The length of the filter paper, which was 57 cm. in these studies, could be increased as desired by coiling the lower end in a small glass support (Figure 1, *b*).

GENERAL PROCEDURE

The sugar solutions were placed on the starting line on the paper with an open loop of platinum wire (*3*) made to deposit 1 μl. of liquid. By alternate spotting and drying, the amount of sugar deposited can be increased without increasing the size of spot. Thus five applications of a 20% solution have given 1 mg. of sugar in a spot about 3 mm. in diameter. When high concentrations of sugar are used in the ascending technique, the starting line should be about 8 cm. above the solvent surface instead of the usual 4 cm. This reduces streaking of the spots by ensuring a slower movement of the solvent by the time it reaches the original spot of sugar. There is better opportunity, therefore, for establishment of equilibrium of the sugars between the stationary and moving phases.

The sheets of paper used in obtaining the data in Tables I and II by the ascending technique were 36 cm. wide and 46 cm. high. The solvent was allowed to advance 28 cm. past the sugar starting line. Equally satisfactory results have been obtained with larger sheets of paper and greater movement of the solvent front. The solvent mixture (200 ml.) in the bottom of the jar was renewed after two developments.

For the descending method, 36 × 57 cm. sheets of paper were spotted with sugar solutions along the 36-cm. side, then split into

two 18 × 57 cm. pieces. The solvent was allowed to travel 50 cm. beyond the starting line, 40 ml. of fresh solvent being used in the boat for each run. To simulate more closely the equilibrium conditions of the ascending experiments, 160 ml. of the solvent mixture were kept in the bottom of the jar. For routine testing this additional solvent can be omitted, although with some solvent combinations, such as the fusel oil–pyridine-water (1:1:1) mixture, the resulting R_F values may be substantially lower. The presence of the aqueous phase of solvent combinations that were incompletely miscible was found to be unnecessary, if not detrimental, for obtaining reproducible results in both the ascending and descending types of chromatography with this apparatus.

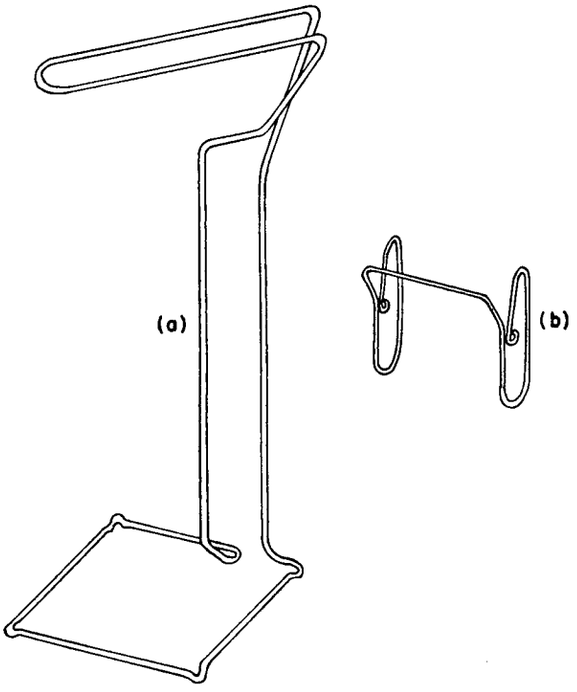

Figure 1. Apparatus for Descending Paper Chromatography

a. Support for glass boat
b. Spiral support for lower end of paper

After development of the chromatograms, most of the solvents listed in Table I were removed satisfactorily from the paper by drying 15 to 20 minutes in the air draft of the hood or 10 minutes in an oven at about 100°. The higher temperature should be avoided as much as possible, particularly in quantitative chromatography, because of the likelihood of some decomposition of the sugars when heated in the presence of pyridine or its analogs (*8*).

The location of the sugar spots on the dried paper was determined by spraying the paper evenly with the dinitrosalicylate reagent just heavily enough to wet through the paper. After a preliminary drying in a hood with the draft on, the paper was heated for 4 to 5 minutes at about 100° in an oven, preferably one with a glass door (*13*). The distances of the estimated centers of the sugar spots from the starting line then were measured to the nearest 0.1 cm.

For the new technique of multiple development, the dried paper was returned to the developing apparatus for a repetition of the passage of the solvent over the paper, this sequence of developing and drying being repeated the desired number of times. The same solvent composition and direction of travel was used in each repetition as in the original development. As a guide in following the progress of the resolution of the spots, a vertical test strip containing one sample of the sugar mixture can be cut off from the edge of the paper after each development and sprayed with the dinitrosalicylate reagent.

RESULTS

The data in Tables I and II, obtained by the ascending method, are representative of the results with various solvents and by both ascending and descending movement of the solvent. The descending runs differed mainly in requiring a shorter time for a given distance of solvent travel. In addition, somewhat elongated spots often were produced, especially with high concentrations of sugar in the spots. This effect caused greater overlapping of spots in chromatograms of mixtures of sugars having very similar R_F values.

The values in Tables I and II were obtained by placing three identical sets of sugar spots on a sheet of paper 36 cm. wide. Development of the chromatogram was at 24 ± 1°, with ascent of the solvent a constant distance of 28 cm. beyond the sugar starting line in each development. The time of solvent travel showed an average deviation of about 1 hour. The average R_F values and distances of movement of the sugars shown in the tables were then calculated from the positions of the three spots of each sugar. The average deviation from the mean was about 0.01 R_F unit. Although these data were obtained with single sugars in the initial spots, essentially the same values were observed when mixtures of sugars were chromatogramed.

For Table I the ratios of R_F maltose to R_F glucose were calculated directly from the original data. They differ in some cases, therefore, from what would be calculated from the rounded-off values of R_F given in the table.

The movement of the sugars has been expressed in Table II as percentage of the distance between the sugar starting line and the constant limit of travel of the solvent front. For single development runs, of course, this is the same as the R_F value × 100. In multiple development runs the "movements" cannot be considered analogous to R_F values. In calculating the percentage movement, the total distance of travel of the sugar spot during the multiple development is divided by the distance of travel of the solvent during a single development, rather than by the total or cumulative distance of solvent travel.

DISCUSSION

The individual sugars nearly always maintain the same order on the basis of R_F values for the solvent combinations shown in Tables I and II, as well as for additional solvents used by the authors and by other workers (*10, 13*). The authors' data also show correlations between chromatographic behavior and structural features such as molecular size, point of linkage between the components of a disaccharide, and configuration of the glycosidic carbon atom. These relationships are useful as a basis for judging what types of sugars are present in a mixture preliminary to isolating and characterizing the components.

The data in Table II show that for the sugars studied, pentoses have higher R_F values than hexoses, and monosaccharides higher than disaccharides. The behavior of mixture A suggests that the R_F value continues to decrease with increased molecular size in a homologous series of oligosaccharides. The 1,4-linked disaccharides move more rapidly than the corresponding 1,6-linked disaccharides. Finally, the α-glycosidically linked disaccharides move more rapidly than the corresponding β-glycosidically linked compounds, although the differences are very small, as in the case of maltose and cellobiose. The R_F relationship between two sugars is maintained when they are converted to glycosides. Thus, glucose has a higher R_F value than galactose, while among the disaccharides the glucoside cellobiose has a higher R_F value than the corresponding galactoside, lactose.

The R_F values of the components of mixture A provide some support for the belief that the mixture contains the homologous series of α-1,4-linked glucose polymers. An essentially linear relationship exists between $\log\left(\frac{1}{R_F} - 1\right)$ and the assumed degree of polymerization. This type of relationship was demonstrated by Bates-Smith and Westall (*2*) for the effect on R_F of the number of like substituent groups on an aromatic nucleus, a series which can be considered analogous to a homologous series. A potentially more useful relationship observed with mixture A is that a nearly straight line is obtained on plotting the logarithm of the

Table II. Movement of Mono- and Oligosaccharides for Single and Multiple Development with Various Solvents

(Movement expressed as percentage of distance to fixed stopping point of solvent front)

	Solvent Composition and Classification											
	Fusel Oil–Acetic Acid–Water 4:1:1 (0.27)[a]		Butanol–Pyridine–Water 3:1:1.5 (0.54)			Butanol–Pyridine–Water 3:2:1.5 (0.65)			Fusel Oil–Pyridine–Water 7:7:6 (0.76)		Fusel Oil–Pyridine–Water 1:1:1 (0.83)	
	Number of Developments											
	1	4	1	2	4	1	2	4	1	4	1	4
D-Xylose	15	42	33	51	74	47	68	84	49	84	48	82
D-Mannose	11	33	27	43	66	41	62	79	42	79	43	78
L-Arabinose	13	38	27	43	66	41	62	79	41	79	41	78
D-Fructose	11	35	26	42	65	40	61	79	41	79	41	78
D-Glucose	8	26	21	36	58	35	56	75	38	75	38	74
D-Galactose	7	24	19	32	51	31	50	70	34	72	35	70
Maltose	3	9	12	19	33	25	41	60	32	64	33	64
Cellobiose	2	8	10	17	30	23	38	56	30	61	31	61
Isomaltose	1	6	9	13	24	19	32	48	26	54	27	54
Gentiobiose	1	5	8	13	23	19	31	49	26	55	26	54
Lactose	1	6	8	13	23	18	30	47	25	54	26	53
Melibiose	1	5	7	12	20	16	27	43	23	51	24	50
Mixture A[b] containing												
DP-1 (glucose)	8	27	22	37	58	35	56	75	39	76	39	76
DP-2 (maltose)	..	8	11	19	33	24	41	60	31	64	32	64
DP-3	..	3	6	10	18	17	28	45	26	53	26	54
DP-4	..	..	..	5	10	10	19	31	20	42	21	43
DP-5	..	..	..	..	5	..	12	20	17	32	17	35
DP-6	..	..	..	..	..	..	..	14	..	27	..	30
DP-7	..	..	..	..	..	..	..	10	..	23	..	26
DP-8	..	..	..	..	..	..	..	..	..	18	..	22
DP-9	..	..	..	..	..	..	..	..	..	14	..	18
DP-10	..	..	..	..	..	..	..	..	..	12	..	14

[a] Solvent classification in terms of ratio R_F maltose–R_F glucose.
[b] For components below underlined value in each column spots overlapped too much to permit reliable estimation of position of their centers. All or most of components moved to some extent, even highest degree of polymerization component being well above starting line after one development with last two solvents.

distance of travel of the component sugar spots against the degree of polymerization. This was true for both single and multiple development.

Resolving Power of Solvents. The distance separating the centers of sugar spots on a chromatogram depends on the difference between the R_F values of the sugars when a given distance of travel of the solvent front is used. As the solvent combinations are changed to alter the R_F values of a pair of sugars, the degree of resolution also changes, as shown in Table I.

The solvents have been arranged here in order of increasing values of the ratio R_F maltose–R_F glucose. As this ratio increases—i.e., the two sugars approach the same rate of movement on the paper—the resolution, or difference in R_F values, of glucose and fructose or of maltose and glucose increases to a maximum, then decreases. This occurrence of a maximum in the degree of separation was observed with other sugar pairs and with a number of solvent combinations in addition to those listed in Table I.

Classification of solvents on the basis of the ratio R_F maltose–R_F glucose proved more satisfactory for bringing out these relationships than did arrangement on the basis of just the R_F values for one sugar. Thus the fusel oil–pyridine-water (1:1:1) mixture would be expected on the basis of glucose R_F value to give separations identical with those for the Chargaff mixture (*4*) of 1-butanol–pyridine-water (3:2:1.5). The observed separations instead are in line with the present arrangement. The use of the ratio minimizes possible effects of differences in temperature and of greater rates of evaporation of some solvent combinations.

The R_F value of a sugar can be varied by using different solvent combinations, as shown in Table I. The use of ternary mixtures with variations of proportions is particularly desirable, as has been suggested also by Jermyn and Isherwood (*10*). The most suitable solvent combinations, from the standpoint of obtaining relatively uniform results, have been those containing an alcohol, a pyridine-type base, and water. The use of acidic mixtures, such as those containing phenol or carboxylic acids, causes exceptional R_F relationships in a few cases. This may be due to selective changes in the proportions of ring and anomeric sugar isomers present. Another complicating factor observed in the use of carboxylic acid–alcohol combinations was change of composition of the solvent during development because of esterification (see also *8*). In the first approach to new problems in the chromatographic separation of sugars it seems advisable, therefore, to avoid the use of such acidic solvent combinations until the inadequacy of the other combinations has been demonstrated.

Only in a very few cases has resolution of certain sugars consistently failed in these studies because of the small differences in R_F values with the solvents used. The members of the mannose-arabinose-fructose group and of the isomaltose-gentiobiose-lactose group were inseparable. Partial separation to give elongated spots occurred with the maltose-cellobiose and the lactose-melibiose pairs. In general, for absolute separation of a pair of sugars into two distinct spots under the conditions used here, their distances of movement must differ by about 3% of the distance between the starting line and the limit of travel of the solvent front. At least for the separation of some or all combinations of mannose, arabinose, and fructose the use of other solvent mixtures, such as phenol-water (*13*), apparently would prove advantageous. In addition, specific color sprays can be used to obtain further evidence of the monosaccharides present.

Multiple Development Technique. The multiple development technique was devised to gain the advantages of a greater effective distance of travel of the solvent front while retaining a shorter length for the paper itself. In addition, all the sugars remain on the paper and the course of their resolution can be followed conveniently by spraying test strips, as indicated in the experimental section. The examples in Table II are typical of the generally better separations obtained with multiple development by either the ascending or the descending technique. In the case of mixture A, values have been omitted when the spots overlapped too much to allow reasonably accurate locating of the centers of the spots. These components had moved, however, as indicated by the presence in the developed chromatogram of a continuous streak down to or above the starting line.

The theoretical distance of separation of two sugars after any given number of developments depends on the total distance of travel of each spot. For the derivation of the mathematical relationships between distance of travel of a sugar and its R_F value, a constant distance of travel, L, of the solvent front beyond the original starting line each time must be specified. In prac-

tice this value is easily held constant by stopping the development when the solvent front reaches a guide line marked on the paper.

The distance which the spot moves during any development, n, will be

$$M_n = LR_F(1 - R_F)^{n-1} \quad (1)$$

The distance of movement in successive developments becomes smaller because, as the spot moves away from the starting line, the solvent front has a shorter distance to travel between the spot and the stopping line, so that the spot is acted upon for a shorter time.

The total distance, M_{total}, which a given sugar would move in n developments is the summation of the distance of travel in each development. This summation simplifies to

$$M_{total} = L[1 - (1 - R_F)^n] \quad (2)$$

The distance of separation of the centers of two sugar spots reaches a maximum as the number of developments is increased. The maximum separation occurs in the development before the one in which the two spots move the same distance. The number of developments, $n_{max.}$, necessary to give the maximum difference of separation of two sugars moving at nearly the same rate, R_F, can be calculated conveniently by the equation

$$n_{max.} = \frac{1}{R_F} - 1 \quad (3)$$

which is the empirically derived limit, as the two R_F values approach each other, of the general relationship

$$n_{max.} = \frac{\log R_{F1} - \log R_{F2}}{\log (1 - R_{F2}) - \log (1 - R_{F1})}$$

Although these equations are derived on a theoretical basis, the experimental values generally were in good enough agreement to justify using the equations as a basis for predicting the approximate behaviors of the sugars on multiple development.

Multiple development causes a flattening of the spots, particularly noticeable with sugars of high R_F, because the edge first reached by the solvent starts moving before the solvent reaches the farther edge. This behavior, although partly counteracted by diffusion, is of benefit in improving the separation of the edges of spots of component sugars in mixtures. This makes multiple development particularly desirable when there are tendencies for the spots to streak because of high sugar concentrations or excessive speed of movement of the solvent over the sugar spots.

Choice of Conditions for Maximum Resolution. The solvent combinations used in these studies have given a well-defined pattern of behavior of a wide variety of sugars. The resulting relationships of resolution of sugars to the solvent ratio R_F maltose–R_F glucose and to the number of developments facilitate the selection of optimum conditions for the chromatographic study of sugar mixtures.

The first step in the separation of a new mixture is a preliminary chromatogram. This will give an indication of the complexity of the mixture and will show the ease of movement of the components relative to the range represented by the sugars in Table II. For this preliminary chromatogram a solvent with an R_F maltose–R_F glucose ratio of about 0.65, such as the butanol-pyridine-water mixture (3:2:1.5), is particularly suitable, because such solvents are best for the sugars of intermediate ease of movement but likely to give usable results with both the faster and slower moving sugars.

The final choice of solvents and number of developments is based on the relative ease of movement of the components and the degree of similarity of their R_F values (resolving power required). The general pattern of optimum conditions for the different sugars is brought out in Table III. The experimentally determined differences in distance of travel of the sugar spots are shown as a function of the solvent (ratio R_F maltose–R_F glucose) used and the number of developments (up to four). The distances of separation were calculated by multiplying the observed distance in centimeters of separation by $100/L$, where L is the constant distance of travel, in centimeters of the solvent front beyond the sugar starting line in each development.

In Table III the best separations were obtained, in general, under the conditions which would be recommended on the basis of the generalizations presented above (indicated by the underlined values). The few apparent discrepancies result from differences that are within the limits of variability of the runs.

In choosing a solvent, advantage is taken of the fact that the resolution passes through a maximum as solvents of increasing ratio, R_F maltose–R_F glucose, are used. This maximum occurs at higher ratio values as the relative ease of movement of the sugars decreases. Thus, for best separation of a pair of chromatographically similar sugars behaving like the pen-

Table III. Effect of Solvent and Number of Developments on Separation of Sugars[a]

(Distance of separation expressed as percentage of the distance between sugar starting line and the stopping point of the solvent front)

	Solvent Composition and Classification											
	Butanol-Pyridine-Water 3:1:1.5 (0.54)[b]			Butanol-Pyridine-Water 3:2:1.5 (0.65)			Fusel Oil-Pyridine-Water 7:7:6 (0.76)			Fusel Oil-Pyridine-Water 1:1:1 (0.83)		
	Number of Developments											
	1	2	4	1	2	4	1	2	4	1	2	4
Mono- and Disaccharides												
Xylose-mannose	6	8	8	6	6	5	6	4	5	5	4	4
Mannose-arabinose	0	0	0	0	0	0	2	1	0	2	1	0
Arabinose-fructose	0	0	1	0	1	0	0	0	0	0	0	0
Fructose-glucose	4	6	7	4	4	4	3	2	4	2	4	4
Glucose-galactose	3	4	7	4	6	5	4	4	3	4	5	4
Galactose-maltose	8	13	18	7	10	10	3	8	8	2	4	6
Maltose-cellobiose	1	2	3	2	3	4	2	1	3	2	1	3
Cellobiose-isomaltose	2	4	6	4	6	8	4	6	7	4	6	7
Isomaltose-gentiobiose	1	0	1	0	1	−1	0	0	−1	0	1	0
Gentiobiose-lactose	0	0	0	0	1	2	0	0	1	0	2	1
Lactose-melibiose	2	2	3	2	2	4	2	2	3	3	2	3
Mono- and Oligosaccharides												
Xylose-glucose	11	15	16	10	12	9	10	7	9	9	9	8
Glucose-maltose	10	17	25	11	16	15	7	12	11	6	9	10
DP-2–DP-3	6	9	15	8	13	15	6	9	11	6	8	10
DP-3–DP-4	..	6	8	6	10	14	4	8	11	6	7	11
DP-4–DP-5	..	..	5	..	7	11	4	7	10	4	6	8
DP-5–DP-6	..	..	..	..	..	6	..	4	5	..	4	5
DP-6–DP-7	..	..	..	..	..	6	..	3	4	..	3	4
DP-7–DP-8	..	..	..	..	..	..	..	4	5	..	4	4
DP-8–DP-9	..	..	..	..	..	..	..	3	4	..	3	4
DP-9–DP-10	..	..	..	..	..	..	..	2	2	..	3	4

[a] Separations are between centers of spots, values being averages from data obtained by both ascending and descending methods with single sugars and mixture A of Table II.
Underlined values show solvent classification that would be recommended for best separations on basis of generalizations in text.
[b] Solvent classification in terms of ratio R_F maltose–R_F glucose.

toses, a solvent with ratio about 0.54 would be used. For sugars behaving like the oligosaccharides, assumed to be DP 7 and 8 in mixture A, a solvent ratio of about 0.8 would be preferable.

The selection of a solvent combination is aided by the fact that nearly the same results are obtained over a moderate range of solvent ratio R_F maltose–R_F glucose. This is of particular value when only one pair of sugars in a mixture have very similar R_F values. The solvent chosen to separate this pair best will generally give very satisfactory separation of the other components of the mixture.

The use of the multiple development technique will depend on the approximate R_F value of the sugars to be resolved, as maximum resolution will be obtained in $\frac{1}{R_F} - 1$ developments. Thus, when chromatograming pentoselike sugars with solvents of R_F maltose–R_F glucose ratio about 0.54, no more than two developments will be profitable. With the oligosaccharides such as DP 7 or 8, on the other hand, multiple development was essential for separation into distinct spots, the positions and separations of which are indicated in Tables II and III.

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Separation and Identification of Some Terpenes by a New Chromatographic Technique

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A chromatographic method for separating terpenes was developed for the determination of the volatile flavoring constituents of citrus fruit. This new technique in organic chromatography has been introduced by using adsorbent-coated glass strips in a manner analogous to paper chromatography. After the mixture had been spotted near one end of the strip, the chromatogram was developed with the aid of capillary attraction by dipping in a suitable solvent. The solvent was then evaporated from the strip and the various zones were indicated by spraying with suitable reagents. On spraying with a fluorescein solution and exposing to bromine vapor, compounds which absorb bromine faster than the fluorescein show up as yellow spots on a pink background. Very unreactive compounds can be located by spraying with a concentrated sulfuric-nitric acid mixture and heating to cause charring of the compounds. The technique can be applied to other types of compounds and is a rapid method of checking solvents and adsorbents for use on larger chromatographic columns. The term "chromatostrip" has been suggested for application to these adsorbent-coated glass strips.

IN THE course of determining the volatile flavoring constituents of citrus fruit, the authors desired to develop a chromatographic method for the purification and identification of terpenes. The small amounts of oil obtainable from the fruit made this almost imperative, and in addition it was desirable, because of the nature of the terpenes, to eliminate any form of heat treatment.

Although a considerable number of papers (*16*, *17*) have been published on the chromatography of triterpenes, very little has been done on the chromatography of the simpler terpenes, possibly because of the lack of a suitable indicating reagent. The work on the simpler terpenes, and most of the work on the sesquiterpenes, has been done by arbitrary separation of fractions of the eluting solvent. Winterstein and Stein (*15*), Carlsohn and Müller (*3*), and Späth and Kainrath (*13*) separated a few of the simpler terpenes by chromatography.

The advent of paper chromatography has seen a great increase in the use of chromatographic techniques. Applications of paper chromatography are limited and it was soon evident that ordinary filter paper was unsuitable for chromatographing terpenes. Impregnation of filter paper with various adsorbents has been used to increase the adsorbing strength of the paper both for organic chromatography (*1*, *4*, *5*, *8*) and for inorganic analysis (*6*, *7*), but is limited by the relatively small number of adsorbents that can be used.

In contrast to paper chromatography, column chromatography has a distinct disadvantage in work with colorless compounds, such as the terpenes, because of the difficulty in locating the zones. Fluorescent compounds can be located with ultraviolet light and the method of Sease (*12*) can be used to advantage for many ultraviolet absorbing compounds.

The authors desired to combine the advantages of paper and column chromatography in order to obtain a rapid chromatographic method to which zone-indicating developers could be

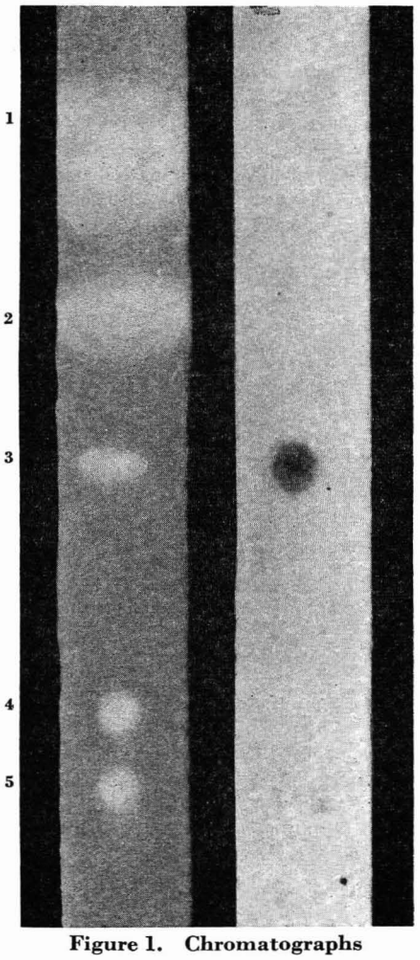


Figure 1. Chromatographs

Left. **Mixture of five terpenes as shown by fluorescein-bromine test**
(From top to bottom)
1. α-Pinene
2. Limonene
3. Terpinyl acetate
4. α-Terpineol
5. Geraniol
Right. **Location of cinnamaldehyde with o-dianisidine reagent**

easily applied. The method of Meinhard and Hall (*9*) on the radial surface chromatography of inorganic ions formed the basis of the present work. Their method was modified by coating the adsorbent, mixed with a binder, on suitable glass strips; the strips were activated and then developed in a manner similar to paper strips in test tubes as used by Flood (*6*) and by Rockland and Dunn (*11*). In order to make the method as universally applicable as possible, Sease's (*12*) idea of mixing two fluorescent inorganic materials—for example, zinc cadmium sulfide and zinc silicate—to the adsorbent was incorporated advantageously.

Because not all of the terpenes absorb in the ultraviolet region and are thus not adaptable to the Sease technique, and because reagents were needed to indicate certain specific functional groups, it was necessary to develop a new series of tests for locating compounds on the chromatograms.

For this new technique, the authors propose the name "chromatostrip."

EXPERIMENTAL

Preparation of Strips. Originally the adsorbent mixture was patterned after that of Meinhard and Hall (*9*) who used 6.2 grams of adsorbent, 3.5 grams of Celite, and 0.5 gram of starch, all of which were heated with 18 ml. of distilled water until the starch had coagulated and the mixture had formed a thick paste. The paste was then triturated with water to a consistency just thin enough to spread on the glass strips. Satisfactory strips could be prepared without the addition of filter aid; this proved desirable because the strips containing Celite were weaker in adsorptive power than the strips containing only adsorbent and binder. Therefore, the mixture contained 19 grams of adsorbent, 1 gram of Amioca starch, and (as fluorescing agent described later) 0.15 gram of zinc silicate and 0.15 gram of zinc cadmium sulfide.

Preparation of suitable strips involved careful attention to the details of preparation. The major difficulties were the cracking of the adsorbent and a surface that was too soft. To solve the difficulties, the following procedure was used:

The specified amounts of material, thoroughly blended while dry, were mixed with 36 ml. of distilled water in a 250-ml. beaker. The slurry was then heated on a water bath held at 85° C. with constant stirring until it thickened (1.75 minutes) and was then held at this temperature for 30 seconds longer with stirring. The beaker was removed from the bath and 2 to 7 ml. of water were added immediately to form a thin paste. The mixture was then spread on glass strips 0.5 by 5.25 inches. (Longer strips can be used to increase the resolution. Two-dimensional chromatography has been employed by coating sheets of glass and developing in the same manner as filter paper.) It is necessary to obtain a smooth surface both for writing and for ease of detection of spots; this can be accomplished by coating the glass while it is held between two glass guides 0.02 inch higher than the glass strip. The strips were then dried in a forced-draft oven at 105° C. for 15 minutes. This procedure resulted in a strip approximately 0.02 inch thick, with a minimum of cracks and a surface hard enough to write on with a blunt pencil. These strips would not crumble under mild handling.

Chromatostrips which were not dried prior to use in a uniform fashion exhibited a marked difference in R_F value (R_F = ratio of distance traveled by a spot to the distance traveled by the solvent). Limonene chromatographed with hexane on strips dried in a desiccator over phosphorus pentoxide at 65 mm. of mercury for 0.5 hour had an R_F of 0.8, whereas this oil had an R_F of 0.4 when dried at 3 mm. of mercury over phosphorus pentoxide for the same length of time. Exposure of strips to atmospheric conditions for short periods of time also increased the R_F of some of the samples. Thus, it was necessary to desiccate the strips in a standard manner and to limit the time in which they were exposed to the atmosphere before use.

The strips were placed in a desiccator over powdered potassium hydroxide and evacuated to 3 mm. of mercury. (Reasons for this desiccant are explained later.) If placed in the desiccator while still warm from the oven, the strips reached equilibrium in 30 minutes. Before the strips were removed, it was essential that the vacuum be broken only with dry air. For this reason, a tube packed with Ascarite was used to admit air to the desiccator. The strips could not be used after exposure to the atmosphere for periods longer than 10 minutes. Following these precautions, it was possible to obtain reproducible R_F values.

For the spot test with a sulfuric–nitric acid mixture, it was necessary to eliminate the starch as a binder because of its reaction with the hot acid mixture. Plaster of Paris (20%) was substituted as the binding agent. In preparing these strips, a small

Table I. Characteristics of Chromatostrips Made with Various Absorbents

Adsorbent Coating	Physical Characteristics of Strip	Resolution of Oils
Magnesium oxide	Soft	None
Alumina	Excellent	Good
Alumina + silicic acid	Excellent	Good
Calcium hydroxide	Soft, crumbly	None
Starch	Good	None
Dicalcium phosphate	Fair	Some resolution
Bentonite	Good	Oils decomposed
Calcium carbonate	Good	Slight resolution
Magnesium carbonate	Fair	Slight resolution
Filtrol	Good	Separation, but oils decomposed
Filtrol X202	Good	Separation, but oils decomposed
Filtrol, Neutral E	Good	Separation, but oils decomposed
Anex	Fair	None
Florisil	Good	Fair separation
Talc	Good	Slight resolution
Silicic acid	Excellent	Excellent

quantity of the dry mixture (enough for two strips) was mixed with sufficient water to make a thin paste, and was immediately spread on the glass strips. These strips were dried at 75° C. and were used as soon as they had cooled.

Method of Chromatography. The sample to be chromatographed was placed as a small dot near the bottom of the strip and pencil lines were made on the adsorbent to indicate the original position of the sample and the desired length of solvent travel. The strip was then placed in a test tube which contained 1.5 ml. of fresh solvent. The strip was removed when the solvent reached the desired height (10 cm.), and the solvent was allowed to evaporate from the strip before the qualitative tests were applied.

Solvents. With silicic acid–coated strips, a search was made for suitable solvents to be used in chromatographing terpenes. The solvents tried were divided into four general classes. (Earlier experiments had shown that the strips could not be placed directly in a solvent containing water, because the adsorbent beneath the surface of the liquid tended to slide off the glass strip. Subsequently, this difficulty was eliminated by placing the strip on a wad of cotton saturated with the solvent.) These four classes are described, together with the solvents falling in each class, as follows:

1. Those that carried all oils to the top of the column (ethyl alcohol, dioxane, diethyl ether, acetone, 1-nitropropane, pyridine, ethyl acetate, methanol)
2. Those that did not move the majority of the oils (hexane, petroleum ether, carbon tetrachloride, carbon disulfide)
3. Those that moved the oils a reasonable distance (chloroform, benzene)
4. Those that interfered with the fluorescein-bromine test (tetrahydrofuran, diacetone alcohol, amylene, ethyl triethoxy silane)

Various mixtures of group 1 with 2 and 3 were investigated, and 15% ethyl acetate in hexane was selected as one of the best solvents for this work.

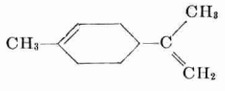
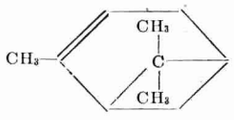
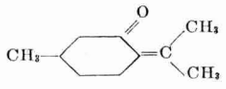
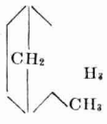
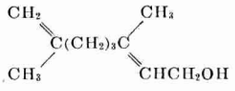
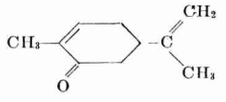
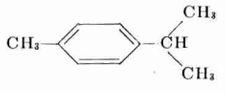
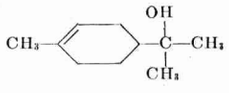
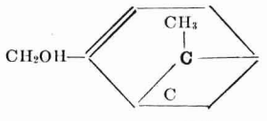
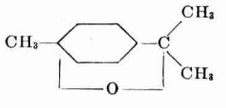
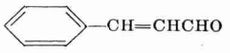
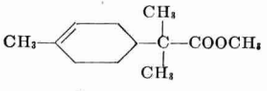
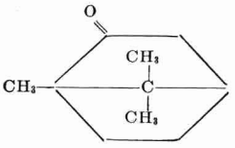
Adsorbents. Of the numerous adsorbents tested (Table I), silicic acid proved to be the best for terpenes. Merck's reagent grade, which had been sifted to pass a 100-mesh sieve, was used in the preparation of the strips for the terpene work.

Color Tests. A wide variety of types of compounds are encountered in the examination of terpenes, so that it is necessary to have several qualitative tests to locate the spots formed on the chromatograms. It is also useful to have, in addition, a variety of tests for specific functional groups. The following tests were worked out and used in the task of locating compounds.

Fluorescein-Bromine. The principle of adding bromine to unsaturated linkages was used in locating a large number of compounds with ethylenic-type double bonds.

The completed chromatogram from which the solvent had evaporated was sprayed

Table II. Reactivity of Compounds with Various Zone-Indicating Tests

Compound	Formula	Ultra-violet	Fluorescein-Bromine	Sulfuric (Concd.)	Sulfuric-Nitric (Concd.)
Limonene		−	+	+ Brown	+
α-Pinene		−	+	+ Brown	+
Pulegone		+	+	+ Yellow	+
Camphene		−	+	+ Brown	+
Geraniol		−	+	+ Purple	+
Carvone		+	+	+ Pink	+
p-Cymene		+	−	−	+
α-Terpineol		−	+	+ Green	+
Nopol		−	+	+ Green	+
1,8-Cineol		−	−	+ Green	+
Cinnamaldehyde		+	+	−	+
n-Capric acid	$CH_3(CH_2)_8COOH$	−	−	−	+
Terpinyl acetate		−	+	+ Brown	+
Camphor		−	−	−	+

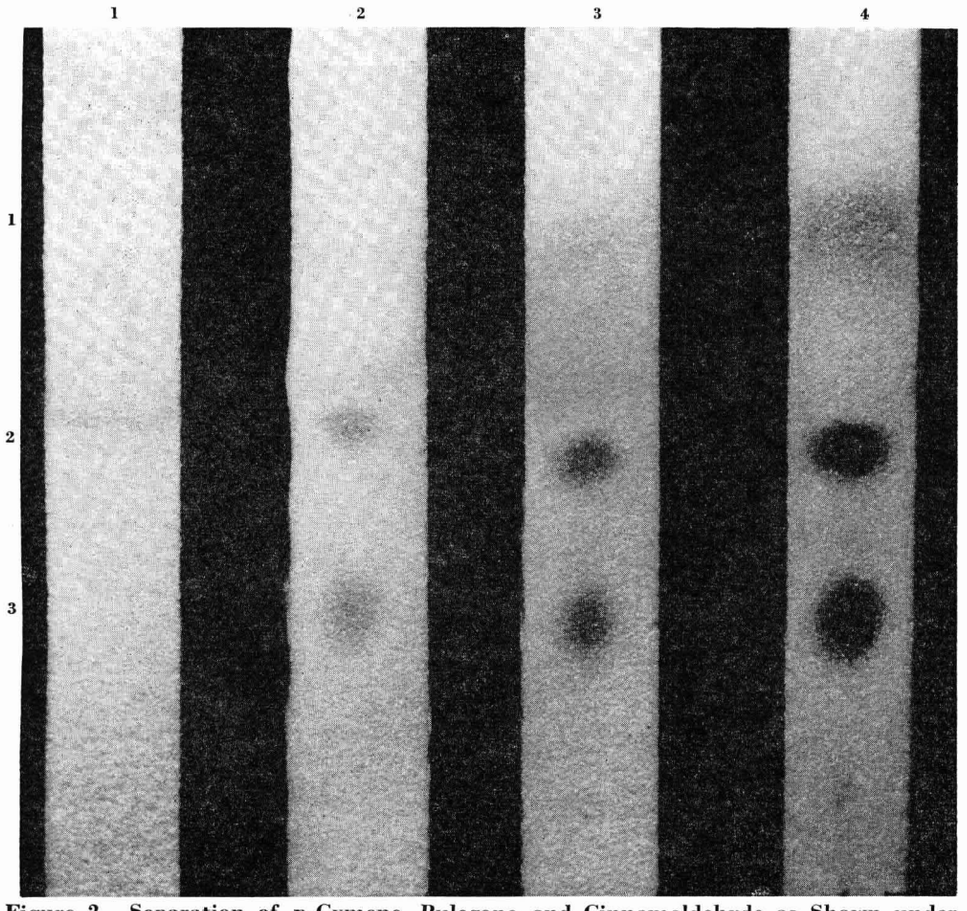


Figure 2. Separation of *p*-Cymene, Pulegone, and Cinnamaldehyde as Shown under Ultraviolet Light on Fluorescent Strips

(From top to bottom)
1. *p*-Cymene
2. Pulegone
3. Cinnamaldehyde

(From left to right)
1. Blank (spot due to traces of impurity in solvent not removed by distillation
2. *p*-Cymene, 143γ, pulegone, 3.3γ, cinnamaldehyde, 3.3γ
3. *p*-Cymene, 358γ, pulegone, 8.2γ, cinnamaldehyde, 9.1 γ
4. *p*-Cymene, 1.4 mg., pulegone 32.8γ, cinnamaldehyde 36.4γ

with a solution of 0.05% fluorescein in water. The strip was then exposed to bromine vapor by blowing gently across the top of a bottle of bromine (a large excess of bromine should be avoided). The fluorescein reacted with bromine to form the red dye, eosin.

Wherever a material was present which could react with bromine, such as an ethylenic double bond, the fluorescein retained its normal yellow color. The location of these compounds was then readily apparent as yellow spots on a pink background (Figure 1). Amounts of some material as small as 1 microgram were detected with certainty on chromatograms with fluorescein-bromine. Table II reveals the types of compounds which can be detected.

Certain precautions must be observed in performing this test. Unless sufficient water is present, the pink color of eosin does not develop uniformly. For this reason, the dilute solution of fluorescein is necessary in order to build up the amount of water on the strip. If desired, a 0.1% solution of fluorescein may be used by first spraying with water. The presence of alkali enhances the red color, while acid prevents its formation. Strips desiccated with phosphorus pentoxide apparently absorbed enough acid vapors to depress the formation of the red color; this difficulty was eliminated by using powdered potassium hydroxide as the desiccant.

Fluorescence. Sease's (*12*) method of fluorescent columns was incorporated by adding zinc cadmium sulfide and zinc silicate to the mixture of starch and adsorbent prior to adding the water. The dried strips gave a bright fluorescence; certain types of compounds could be detected as dark spots when viewed under ultraviolet light (Figure 2). As suggested by Sease, the source of ultraviolet light was important in viewing these chromatograms. Unless ultraviolet light of short wave length was used, no spots were discernible. A Mineralight (Short wave Model SL 2537) gave light of satisfactory wave length for this work. Table II indicates the types of compounds detected by this means.

***o*-Dianisidine.** Aldehydes can be detected as colored compounds with *o*-dianisidine (*14*). Cinnamaldehyde was detected by spraying with a solution of *o*-dianisidine in glacial acetic acid.

Sulfuric Acid. Certain compounds resisted all means of detection because of the absence of reactive groups within the molecule. To detect these compounds, concentrated sulfuric acid was sprayed on the developed chromatogram. This reagent was used in locating 1,8-cineol. Table II indicates the color reactions and types of compounds detected by this reagent.

Special equipment was needed to spray a reagent as corrosive as concentrated sulfuric acid. The spraying was done with an all-glass sprayer built in the laboratory (Figure 3). A small stainless steel booth for spraying was constructed to protect the hood, and the sprayer was manipulated behind a glass window.

Bromocresol Green. Ramsey and Patterson (*10*) have developed a method for the detection of acids on silica gel columns by incorporating bromocresol green in the adsorbent. Acids were detected on the strips by spraying with a solution of 0.3% bromocresol green in 80% by volume methanol, to which had been added 8 drops of 30% sodium hydroxide per 100 ml. The acid appeared as yellow spots on a green background.

Sulfuric–Nitric Acid Mixture. Camphor was so unreactive that it was necessary to develop a special test to indicate the location of this compound. This was accomplished by spraying with concentrated sulfuric acid to which 5% concentrated nitric acid had been added. The chromatograms were then heated on a strip of glass cloth, face down, on top of a hot plate which was turned to full heat and registered approximately 500° C. After the acid fumes had ceased coming off, the glass strip was carefully lifted from the adsorbent and the latter was turned over by means of the glass cloth. The compound locations were observed as black spots on a white background.

Chromatography of Terpenes. Fifty-eight samples of different commercial oils were chromatographed with five solvents on fluorescent silicic acid–coated strips. By this series of tests it was hoped to test the applicability of the technique and to select

a variety of samples of good purity on which sensitivity and R_F values could be determined. Of the 58 samples chromatographed, two were considered to be sufficiently pure; the other 56 oils gave chromatograms on which 2 to 9 spots were apparent when viewed under ultraviolet light and treated with fluorescein and bromine.

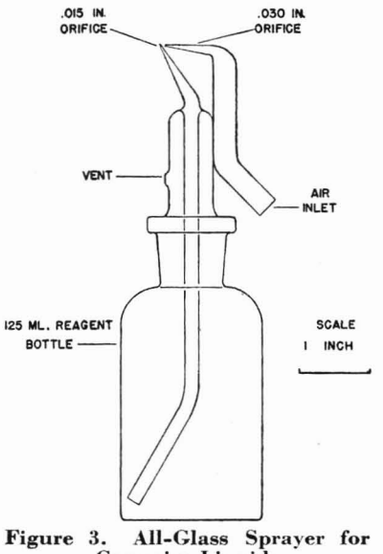


Figure 3. All-Glass Sprayer for Corrosive Liquids

Fourteen compounds were then purified in this laboratory by various techniques. The method of purification and an indication of the purity of these samples are given in Table III. All fractional distillations were made with a Podbielniak Hypercal column. Many different types of compounds were selected in order to indicate the wide range of usefulness of both the technique and the various spot-indicating reagents.

Each of the pure samples was chromatographed on fluorescent–silicic acid strips. Five solvents were used to characterize the oil: hexane (boiling point 65° to 69° C.), carbon tetrachloride, chloroform, benzene, and ethyl acetate in hexane (15% by volume). Sensitivity to the various color tests was determined by measuring the sample applied to the adsorbent, pure or in a suitable solvent, from a calibrated capillary pipet. (The ordinary mercury piston-type microburet could not be used because the oils in contact with the mercury caused the mercury to fall out of the capillary. A calibrated capillary tube fitted with a syringe to control the column of liquid by air displacement was used.) The quantity of sample applied was reduced in systematic fashion until the chromatogram failed to give the color test in question. The last detectable concentration was called the sensitivity limit. On each set of five chromatograms, a control chromatogram of limonene chromatographed with hexane was run in order to make sure the strips were dried properly and to afford a reference for comparing R_F values. A minimum of five samples in each solvent was run to determine the R_F value. Samples diluted with ethyl alcohol tend to have their position on the chromatogram distorted by the alcohol. This phenomenon was not observed when hexane was used as the diluent. On some chromatograms in which the concentration of the oil was high, the R_F was higher than on similar chromatograms with smaller amounts of oil. This was presumed to be caused by a dilution of the ascending solvent by the sample. When the concentration of the sample was high, this dilution was apparently enough to cause a change in the characteristics of the solvent. The results of the sensitivity determinations and the R_F of the terpenes in the various solvents are given in Table IV.

DISCUSSION

By using the technique described herein, a sample of oil can be rapidly chromatographed and many conclusions concerning purity and adulteration can be established at once. Because a

Table III. Preparation of Pure Compounds

Limonene. By fractionation from grapefruit peel oil
B.p. at 8 mm.: observed, 54° C.; literature, 53.35° C.

α-Pinene. By fractionation of a commercial sample
B.p. at 30 mm.: observed, 60.5° C.; literature, 60.5° C.

Pulegone. By fraction from oil of pennyroyal
B.p. at 7 mm.: observed, 86.5° to 89° C.; literature 87.4° C.

Camphene. Commercial sample. M.p.: observed, 45° to 49° C.; literature 49° to 52° C.

Carvone. Purified by forming H_2S addition product and then steam distilling. M.p.: observed, 219° to 224° C.; literature, 222° to 224° C.

Geraniol. By forming $CaCl_2$ addition product from commercial sample and then steam distilling

p-Cymene. Commercial sample (Paragon)

Cineol. Fractionated from oil of Cajeput (b.p. at 9.5 mm.: observed 56.4° C.; literature 54.2° C.), and then further purified by recovery from resorcinol addition product

α-Terpineol. Recrystallized commercial product from hexane, m.p. 34–34.5° C.; literature 35° C.

Nopol. Fractionated, b.p. 92.6° C. at 4 mm.; literature 71° C. at 1 mm., 98° C. at 5 mm.

Terpinyl acetate. Prepared from α-terpineol and acetic anhydride by method of Boulez (*2*)

Cinnamaldehyde. Commercial sample (Paragon) by recovery from sodium bisulfite addition product

n-Capric acid. Recrystallized from acetone, m.p. 33–34° C.; literature 31.3° C.

Camphor. Sample used for molecular weight determinations, m.p. 178.8° C.; literature 178.8° C.

Table IV. Chromatography of Pure Compounds

Compound	R_F Values: Hexane	Carbon tetrachloride	Chloroform	Benzene	15% ethyl acetate in hexane	Control, limonene in hexane	Limit of Sensitivity, γ	Color Test
Limonene	0.41	0.37	0.93	0.96	0.66	...	37	Fluorescein-bromine
α-Pinene	0.83	0.89	0.95	0.96	0.83	0.50	37	Fluorescein-bromine
Pulegone	0.01	0.01	0.09	0.07	0.49	0.51	4	Ultraviolet and fluorescein-bromine
Camphene	0.74	0.82	0.92	0.94	0.79	0.47	200[a]	Fluorescein-bromine
Geraniol	0.00	0.00	0.05	0.05	0.21	0.46	1.5	Fluorescein-bromine
Carvone	0.00	0.01	0.07	0.04	0.45	0.47	0.4	Ultraviolet
							8.0	Fluorescein-bromine
p-Cymene	0.38	0.56	0.94	0.95	0.60	0.43	100[b]	Ultraviolet
α-Terpineol	0.00	0.00	0.05	0.03	0.24	0.43	4.0	Fluorescein-bromine
Nopol	0.00	0.00	0.11	0.06	0.27	0.53	1.0	Fluorescein-bromine
1,8-Cineol	0.01	0.02	0.12	0.06	0.48	0.48	0.6	Concd. sulfuric
Cinnamaldehyde	0.00	0.00	0.09	0.06	0.31	0.48	0.3	o-Dianisidine
Terpinylacetate	0.00	0.00	0.26	0.25	0.50	0.50	1.0	Fluorescein-bromine
n-Capric acid	0.00	0.00	0.07	0.07	0.43	0.55	4.0	Bromocresol green
Camphor	0.00	0 00	0.28	0.22	0.56	0.67[c]	0.8	Concd. sulfuric-nitric

[a] Sensitivity of detection 15γ with sulfuric-nitric acid.
[b] Sensitivity of detection 30γ with sulfuric-nitric acid.
[c] Compound run on strips with plaster of Paris instead of starch as a binder; thus, R_F value for limonene control has a different value.

large number of terpene samples have been investigated and the great majority of them (56 out of 58) have been found impure by examination of chromatograms in five solvents, much of the tedious physical and chemical examination of the sample can be eliminated. The oils examined gave no sign of decomposing or isomerizing on silicic acid. Silicic acid is an excellent adsorbent for this work, because it resolves compounds of very similar type, as illustrated in Table IV.

The identification of terpenes should be facilitated by application of the principle of characteristic R_F values to the chromatographed samples. In identifying constituents of natural products where the amounts of fractions concerned may be small, the volume of material necessary to secure identification can be substantially reduced. Amounts of oil as small as 0.5 microgram have been detected. Some of the color tests reported here have useful implications for indicating structure—for instance, from Table II it is evident that the fluorescein-bromine test reveals the possible presence of ethylenic-type double bonds. Other indications of structure are obtained from a study of Table II, and many other tests can be applied which have specificity for certain types of structures.

As a chromatographic method aside from the advantages to terpene chemistry, many features can be pointed out.

The chromatostrip combines some of the advantages of paper and column chromatography. It adds the rapidity (a strip may be run in 0.5 hour) and the ease of spot development of paper chromatography to the wide range of adsorbents of column chromatography.

The method can be used for rapidly checking solvents and adsorbents for larger columns, because the results obtained are comparable. Although small conventional columns can be used for the same purpose, the proposed method is more rapid inasmuch as one man can easily run a set of 40 strips in an hour's time.

More drastic reagents can be applied to locate compounds than are permissible with paper strips.

"Wet tails" (irregular flow of solvent up side of paper due to touching of the glass wall) and similar annoyances of the flexible paper are avoided.

It is a microchromatographic method and is much more convenient than a packed column for micro work.

The use of concentrated sulfuric acid and concentrated sulfuric-nitric acid mixture for elucidating positions of organic materials on a chromatogram eliminates the uncertainty usually associated with less drastic revealing agents. These reagents have been used to indicate traces of impurities in some terpenes which were thought to be pure by reason of physical constants and chromatography with other compound-indicating tests.

The spray gun used for all of the work except for the sulfuric acid was an artist's air brush.

As shown by Meinhard and Hall (*9*), the completed chromatograms could be stripped off on Scotch tape and pasted on suitable cards for filing and reference purposes. This was not possible for strips sprayed with cold sulfuric acid, but the chromatograms which were heated after spraying with acid could be saved in the same manner.

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Automatic Fraction Collector for Chromatographic Separations

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A METHOD has been developed recently for the chromatographic separation of sugars on charcoal (*1*). For application of the method to separations on a larger scale, an automatic apparatus has been devised which feeds the mixture and the developers in succession to the top of the chromatographic column and collects each effluent in separate receivers. The device has performed well in the fractionation of hydrolyzates of guaran, xylan, and starch, and may have general application for chromatographic separations.

As shown in Figures 1 and 2, the apparatus consists of containers for the solutions to be fed to the column, receivers for the effluents, solenoid valves operating in pairs so that when energized one container and the corresponding receiver are connected to the column, an electronic control unit, a float chamber, a light source, a photoelectric unit, and a vacuum pump. The details of the control circuit and a list of parts are given in Figure 3. All the materials are readily available and easily assembled. The unit can be built for approximately $200, exclusive of labor.

OPERATION OF CIRCUIT

The apparatus is readied for operation by filling the containers, connecting the receivers to the distributor, and inserting a previously packed and wetted column. The space in the tube above the adsorbent must be completely full of liquid to ensure a proper start.

To initiate the operating cycle the main power switch, SW_1, is placed in the "on" position, the reset button, SW_2, is pressed, and the momentary contact switch, SW_3, is closed. The operation of the apparatus is fully automatic from this point. After a delay of 70 seconds, the first solenoid valve in the upper bank is energized, allowing the solution in the first container to fill the float chamber and flow to the top of the column. At the same

Chromatographic separation of materials in sizable quantities involves the handling of large amounts of adsorbents and solvents. In order to reduce the labor required in such a preparative procedure, an electronically controlled apparatus has been devised to feed different developers to a column and collect the effluents in separate receivers. Essentially, the apparatus consists of a photoelectric system, controlled by a float, which operates successive pairs of solenoid valves through the medium of a stepping relay. The developers are passed through the column in sufficient volume and in proper sequence to effect the desired separation. The apparatus has been used for the fractionation of hydrolyzates of guaran, xylan, and starch. It may have general application to chromatographic separations.

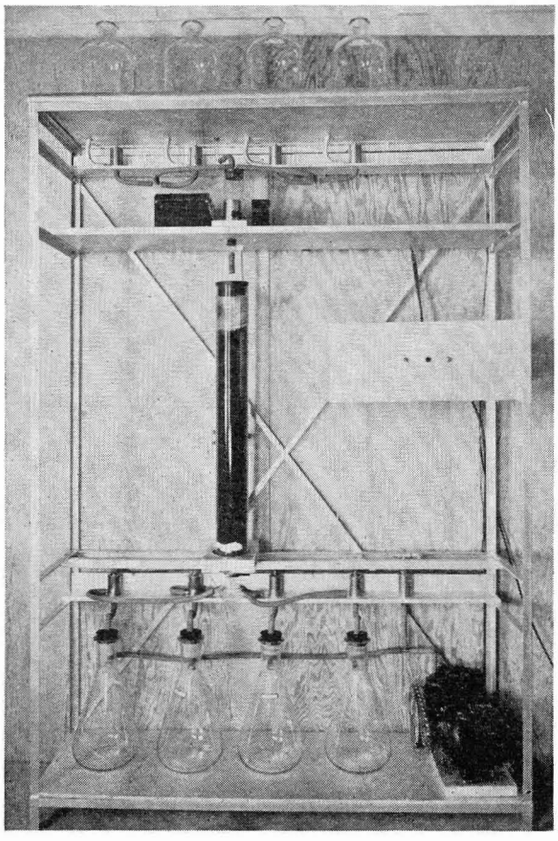

Figure 1. Automatic Fraction Collector

time, the first solenoid valve of the lower bank is energized, connecting the first receiver to the effluent distributor and applying vacuum to the bottom of the column. Thus the solution in the first container passes through the column and is collected in the corresponding receiver. When all this solution has been applied to the column, the float drops and cuts off the light beam. This initiates the switching cycle, which consists of turning off the light source, de-energizing the first pair of solenoid valves, energizing the next pair of valves, and turning on the light source after the float has risen to the top of the float chamber.

The solution in the second container is applied to the column and the effluent is collected in the second receiver. When the float again interrupts the light beam, the switching cycle is repeated. The apparatus ceases operation when all the solution in the last container has passed through the column. It is not required that all containers be used during each run, but only that the developers be placed in successive containers. If, for example, only the first three containers are utilized for a particular run, the apparatus will cease operation 120 seconds after all of the third solution has been fed to the column.

Control of the light source in the manner described is desirable in order to prevent the operation of the photoelectric cell during the time that the float is rising. Thus any bobbing or tilting of the float in this period will not reactivate the circuit.

Although the described apparatus has four containers and four receivers, the number may be increased by the addition of solenoid valves in pairs. These pairs of valves are connected to successive positions of the stepping relay in the manner shown in the circuit diagram. At the same time, the number of successive positions in which the power relay, R_7, is energized must be increased by the number of pairs of valves added. The other modifications include the addition of containers, receivers, and connections to the effluent distributor.

The apparatus will cease to operate in the case of interruption of the current supply or energization of the stepping relay for

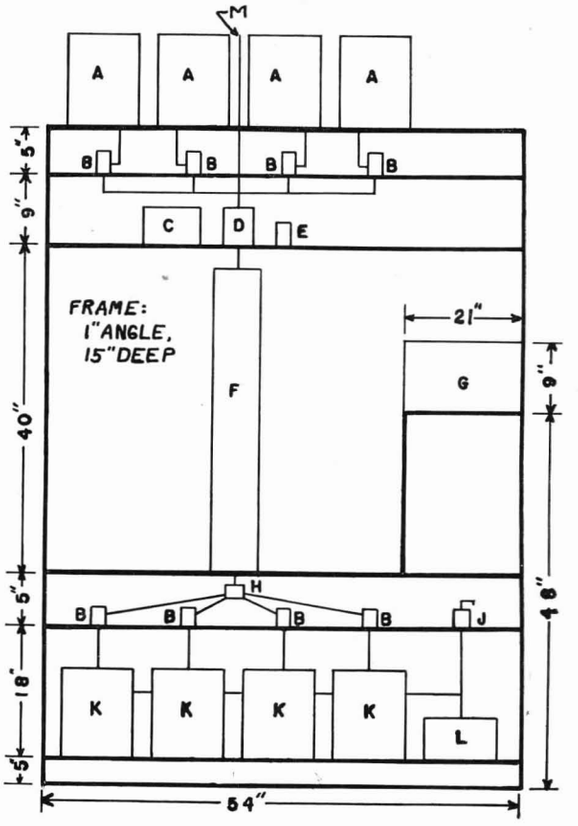

Figure 2. Schematic Arrangement of Components of Automatic Fraction Collector

A. Aspirator bottles
B. V5-2 solenoid valves, Skinner Chuck Co.
C. Photocell unit, Detect-O-Ray Corp.
D. Float chamber, 59 × 200 mm.; float, stainless steel, 2-inch diameter × 3 inches, W. H. Nicholson and Co.
E. Light source, 15-watt
F. Column
G. Control unit
H. Effluent distributor, see Figure 4 for details
J. V5-O solenoid valve, Skinner Chuck Co.
K. Effluent receivers
L. Vacuum pump
M. Air vent

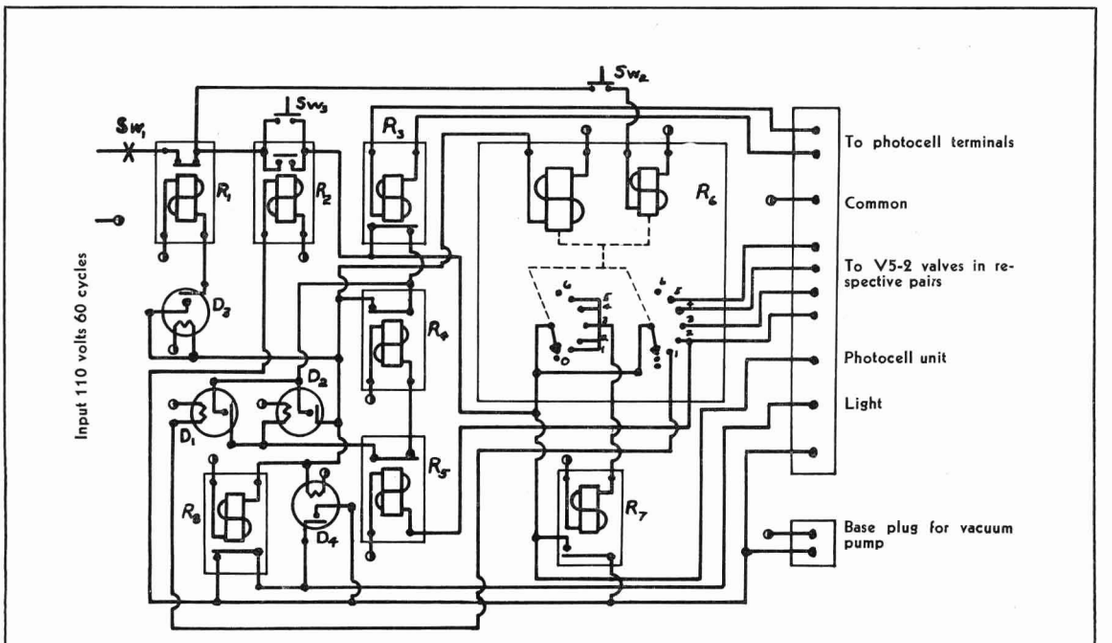

Figure 3. Circuit Diagram of Control Unit

SW_1. Toggle switch, rated 12 amperes at 125 volts
SW_2. Momentary contact switch, rated 3 amperes at 125 volts
SW_3. Momentary contact switch, rated 12 amperes at 125 volts
R_1. PR4A, heavy duty power relay, Potter and Brumfield
R_2. PR3A, heavy duty power relay, Potter and Brumfield
R_3. L-205 power relay, Detect-O-Ray Corp.
R_4, R_5, R_8. MR2A, medium duty power relay, Potter and Brumfield
R_6. Series R stepping relay, 24 positions, with electrical reset and two gang switch, Guardian Electric Manufacturing Co.
R_7. MR1A, medium duty power relay, Potter and Brumfield
D_1, D_4. 115N010 thermostatic delay relay, 10-second delay, Amperite Co.
D_2. 115N060 thermostatic delay relay, 60-second delay, Amperite Co.
D_3. 115N0120 thermostatic delay relay, 120-second delay, Amperite Co.

more than 120 seconds. In the latter case, a safety device consisting of a delay relay, D_3, and a power relay, R_1, will operate to interrupt the current supply, thereby avoiding damage to the stepping relay which is rated for a maximum energization period of 5 minutes. This safety device will operate if the light beam

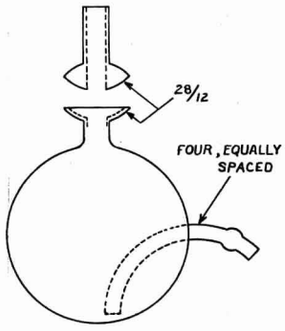

Figure 4. Details of Effluent Distributor

is not restored within 120 seconds of the beginning of each switching cycle because of failure of the lamp filament, of the photoelectric system, or of the float to rise.

APPLICATION OF APPARATUS

The data necessary for the fractionation of a large amount of sugar mixture are obtained by a preliminary small scale experiment using a 44 × 265 mm. column of adsorbent. The weight of material which can be adsorbed and the volume of each of the developers required for the fractionation are determined. The large scale separation is carried out with a column which must have a ratio of length to area at least equal to that of the small test column. When this condition is met, the larger column is operated on the basis of the ratio of its volume to that of the test column. The weight of material which can be adsorbed is equal to the weight determined for the test column multiplied by the adsorbent volume ratio. This same ratio determines the volume of each developer required.

For use with the apparatus, glass tubes ranging from 64 × 565 mm. to 80 × 920 mm. fulfill the required conditions of length and area.

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Stable Internal Standard Flame Photometer—Correction

In the article on "Stable Internal Standard Flame Photometer" [Fox, C. L., Jr., ANAL. CHEM., **23**, 137 (1951)] there is a misprint in Table II, Working Standards for Photometer. The heading for the first column should be Ml./l.

New Type of Chromatographic Column

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In the course of investigations on the volatile flavoring constituents of citrus fruits, it became necessary to chromatograph certain fractions in quantities large enough to perform additional tests. To accomplish this a chromatographic column was developed which was self-supporting and was therefore not encumbered by a containing envelope. The column for which the name "chromatobar" has been suggested, was made from a desired adsorbent and plaster of Paris. After development, the location of the resolved zones could be easily determined by spraying on the sides of the column a suitable reagent, which could be scraped off before recovery of the resolved compounds. A device is described for distributing solvent to the column for development. The operation of the column was demonstrated with some terpenes. This new chromatographic device has many advantages over conventional chromatographic columns, in addition to the convenience of locating resolved zones.

IN THE course of work on the isolation and identification of the volatile flavoring constituents of citrus juices, it became necessary to use chromatographic procedures for the separation of certain terpenes. The use of the adsorbent-packed glass tubes common in chromatography on the colorless terpenes was difficult, because suitable methods for locating these compounds on a developed column were not available. Arbitrary cutting of the column, location by fluorescence, extrusion from the glass envelope and brushing on of an indicating reagent (*4*), and detection by means of strongly fluorescent columns according to the method of Sease (*3*) fell short of satisfying the need for a more convenient and accurate method. As part of the solution to this problem, the "chromatostrip" method of Kirchner *et al.* (*1*) was developed.

Although this method was successful in the isolation and identification of colorless materials, a column of larger dimensions was needed so that larger quantities of material could be chromatographed and recovered from the column for further confirmatory tests. Paper chromatographic methods have been applied to the isolation of larger quantities by Mitchell and Haskins (*2*). It appeared likely that the principles and techniques of the chromatostrip could be applied to the making of a larger column, which would be free of a containing glass envelope and the consequent disadvantages of columns packed in glass tubes.

This paper describes the preparation of a rigid, self-contained column, and its application to the separation of some terpenes. The name "chromatobar" is proposed for this type of column.

PREPARATION OF COLUMN

The column is made from a mixture of a suitable adsorbent with calcium sulfate hemihydrate (plaster of Paris).

For a column 1 inch square by 10 inches long (2.5 × 25 cm.), 18 grams of calcium sulfate hemihydrate and 72 grams of the desired adsorbent are weighed into a bottle and shaken vigorously until thoroughly blended. The mixture is then poured into a beaker, 135 ml. of water are added all at once, and this mixture is stirred vigorously and poured into a previously prepared mold. The amount of water to be added to a batch of mixture must be determined empirically for each different lot of an adsorbent and each different adsorbent. The final slurry should be just liquid enough to pour easily. The above-mentioned quantity of water is for a silicic acid adsorbent. The time between the addition of water and pouring of the slurry into the mold must be kept to a minimum (30 seconds), as the slurry sets very rapidly. After approximately 5 minutes, the bar can be removed from the mold. The mold consists of a piece of metal formed into the shape of the column desired. A glass rod located in the center of the bar lengthwise must be used to give added strength to the column. For the preparation of 1 × 1 × 10 inch columns, a piece of metal 10 inches long and 3 inches wide is bent to form a trough 1 inch wide, 1 inch deep, and 10 inches long. The ends of the trough are made from stiff cardboard cut to a 1-inch square with a hole in the center to hold the glass rod. The trough is lined with waxed paper and the glass rod is inserted through the holes in the ends, so that approximately 6 inches of the rod extend from the head of the mold and 0.125 inch from the base.

According to the adsorbent being used, various methods of drying or activating can be employed. With silicic acid columns, the bar is placed in a forced-draft oven at 75° C. for at least 8 hours. After drying, the column is cooled and stored in a desiccator over potassium hydroxide at 3-mm. vacuum.

Columns have been made of silicic acid, alumina, magnesium oxide, Anex, and Filtrol. The bars are rigid, maintain their shape, and can be handled without breaking.

METHOD OF CHROMATOGRAPHY

These rigid columns are used for chromatography in much the same way as the chromatostrips, but some precautions and special techniques are necessary.

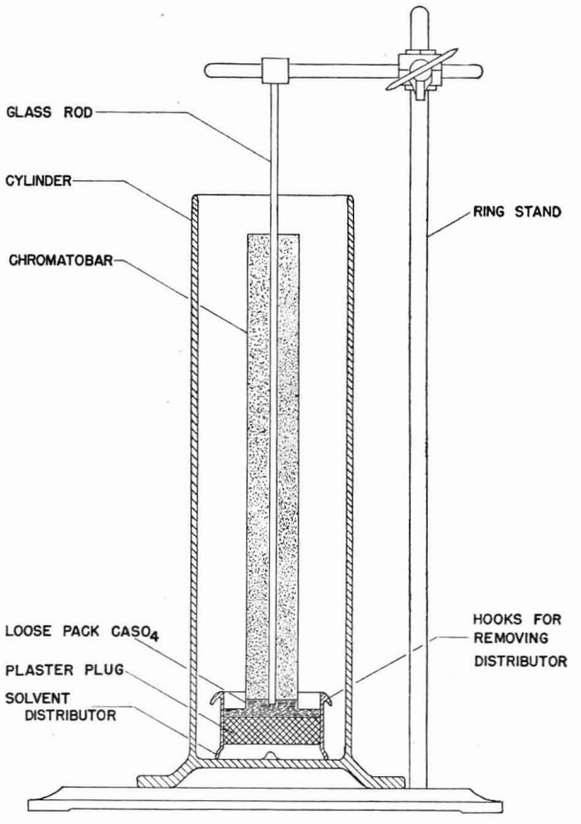

Figure 1. Apparatus for Developing Chromatobar

The column is removed from the desiccator, the entire surface is lightly rubbed with fine sandpaper to remove irregularities, and the column is then placed between two blocks of wood whose end surfaces are smooth and perpendicular to their sides. With a piece of sandpaper stretched across a straight surface, the end of the bar is sanded lightly, using the blocks of wood as guides, until a smooth surface at right angles to the sides of the bar is obtained. The end of the bar is first wet with about 1 ml. of the solvent and the material to be chromatographed is placed on the end of the bar by spreading evenly from a pipet. Dilute solutions of the materials to be chromatographed should be used to ensure even distribution across the surface of the bar. The bar with the material to be chromatographed is then ready for use.

Figure 2. Chromatograph of Crude Preparation of Isoeugenol on Fluorescent Column

Photographed on ultraviolet light
1. Solvent front
2. Isoeugenol
3, 4, 5, 6. Unknown constituents

Figure 3. Chromatograph Showing Separation of (1) α-Pinene, (2) Terpinyl Acetate, and (3) α-Terpineol

Compounds located by fluorescein-bromine test

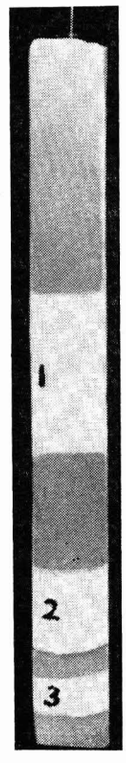

Figure 4. Separation of (1) Limonene, (2) Terpinyl Acetate, and (3) α-Terpineol

Compounds located by fluorescein-bromine test

The column is developed by means of a solvent distributor. This distributor consists of a 2-inch section of glass tubing of suitable size (2 inches for a 1-inch bar), which is indented in three places at the bottom to allow free access of solvent and has three glass hooks at the top for convenience in removing it from the containing vessel. About half of the tube is filled with the same mixture of calcium sulfate hemihydrate and adsorbent used in the bar to form a solid base and dried as in the preparation of the bar. Loose, easily packed material (such as calcium sulfate) is placed on the surface of this plaster plug and very lightly firmed with a wooden rod. The solvent distributor is placed in the bottom of a cylinder and the column is gently but firmly pressed into the cake of loose material on the distributor. The column is held firmly in place with slight pressure by means of a clamp on the glass rod in the center of the column. A small tamping rod is then used to press down the loose material around the bar and brush away any excess.

The solvent is poured down the sides of the cylinder until a suitable amount is present, but is not allowed to overflow the sides of the distributor. The solvent rising through the solvent distributor and the column by capillary attraction advances up the column in an even manner. When the solvent has reached the desired level, the bar is removed and examined by any of the techniques available for detecting the compounds chromatographed. If necessary, it can be returned to a new solvent for further development after the detecting reagent has been scraped off. Figure 1 is a drawing of the assembly used for the chromatography.

SEPARATION OF SOME TERPENES

The column can be used to separate materials in sufficient quantities to be examined by other means. The separations described were made on silicic acid (80 mesh or smaller) columns of 1-square-inch cross section and 10 inches long. Because the analogy to the chromatostrips is very close, numerous strips should be run to permit selection of the best adsorbent and solvent conditions for the desired separation.

In one experiment, 100 mg. of a crude preparation of isoeugenol were dissolved in 1 ml. of ethyl acetate and applied to the column. The column was developed with 15% ethyl acetate in hexane. Approximately 125 ml. of solvent were required to develop a 1-square-inch column 10 inches long. The time of development for such a column was about 4 hours. Figure 2 is a photograph of the resulting column, showing the six distinct bands that were formed. The column in this instance was made with 0.075% zinc cadmium sulfide and 0.075% zinc silicate incorporated in the adsorbent (*3*) and the bands were then visible in ultraviolet light.

In a second experiment, 17 mg. of α-pinene, 10 mg. of α-terpineol, and 10 mg. of terpinyl acetate in 1 ml. of hexane were added to the bar and developed with 15% ethyl acetate in hexane. Figure 3 is a photograph of the resulting column after it had been sprayed with fluorescein and treated with bromine vapor (*1*). The three compounds are visible as bands of yellow on a pink background.

When a mixture of limonene, α-terpineol, and terpinyl acetate was chromatographed on a chromatostrip, it appeared that these three compounds were separable with 15% ethyl acetate in hexane. However, when a mixture of 20 mg. of limonene, 20 mg. α-terpineol, and 20 mg. of terpinyl acetate was chromatographed on a column with 15% ethyl acetate in hexane, good separation of limonene and terpinyl acetate was not obtained. As experiments showed that α-terpineol and terpinyl acetate do not move when chromatographed with hexane whereas limonene is moved to an R_F value of 0.4, the above mixture of compounds was placed on a column and developed with hexane until the solvent had traveled $^2/_5$ of the column. The bar was then transferred to another solvent distributor and the development finished with 15% ethyl acetate in hexane. This resulted in a good separation of the three components, as shown in Figure 4.

DISCUSSION

The rigid, envelope-free column was designed to overcome one of the disadvantages of the column packed in glass—i.e., the difficulty of locating colorless compounds without previous extrusion of the column. Large quantities of material can be isolated from this column, and colorless compounds can be detected by the techniques of paper or chromatostrip chromatography. The square bars are very convenient for locating bands, because each of the four sides can be used for spraying a different reagent. In one experiment, citral was chromatographed and sprayed with fluorescein-bromine on one side and with *o*-dianisidine on the other side. By this means the position of the aldehyde with ethylenic bonds was determined and it was thus differentiated from other aldehydes present as impurities. The surface of the bar can be easily scraped free of the sprayed material and the product recovered without the presence of interfering substances.

Use of the chromatobar eliminates the task of packing a satisfactory column in glass tubes—a particularly difficult task when columns of large size are desired. The columns used in the illus-

trations for this paper were 1 square inch in cross section; columns 2 and 4 inches square have been made without difficulty. When shaved with a knife, these columns show no evidence of nonuniformity in packing.

By use of the capillary-ascent method of solvent travel combined with the rigid characteristics of the column, it is a matter of small concern to transfer the bar from one solvent to another. A preliminary examination of the position of the bands can be made by spraying an appropriate reagent on the column, scraping the reagent off the sides, and then returning the bar to that solvent or a new solvent combination for further development. This is a distinct advantage over a packed chromatographic column, where further development is impossible once the column has been removed from the glass tube. Although conventional chromatographic borosilicate glass tubes can be used for fluorescent chromatography with the longer wave-length ultraviolet light, the shorter rays (230 to 290 mμ) needed for excitation of the zinc silicate are absorbed by this glass. Therefore, this method is particularly adaptable to the use of the ability of compounds to absorb ultraviolet light, because columns made fluorescent with zinc cadmium sulfide and zinc silicate can be observed without being removed from the solvent and without interference due to absorption of the short wave-length ultraviolet light by a glass envelope.

A number of developed columns have been examined for uniformity of the bands through the interior of the column. Successive small layers have been removed from the surface and the new surface examined by spraying or by observation in ultraviolet light. The zones have been found to be uniform throughout the cross section of the column. The zones have been more uniform than have the zones of columns packed in glass, because the solvent in these columns tends to travel more rapidly along the glass surfaces than through the adsorbent. Occasionally a column will crack in a direction perpendicular to the supporting glass rod during the drying period. This defect can be remedied by gently pressing the bar together; columns with such cracks have been used without any effect on the movement of solvent or compounds.

Round bars have been made with thin plastic casings as molds; however, the square bars are more convenient for applying color-developing reagents by means of sprays. Solvents containing water have been used to develop columns; although they impart fragility to the column, such columns can be successfully handled. These columns can be accurately cut with a coping saw and areas of interest removed and extracted with suitable solvents.

ACKNOWLEDGMENT

The authors are particularly grateful to George J. Keller of this laboratory for the preparation of the drawing of Figure 1.

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Compound Types in Gasoline by Mass Spectrometer Analysis

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A NUMBER of laboratories throughout this country use commercial model mass spectrometers to obtain high precision analyses on a wide variety of organic compound mixtures (*6, 7, 10, 12, 14*). Notable success has been achieved in the field of hydrocarbon analysis, where very small samples containing up to thirty components can be analyzed in 1 or 2 man-hours (*2, 3, 16*). In view of the speed, accuracy, and small sample requirements of these methods it appeared highly desirable to extend the scope of the mass spectrometer to compound-type analysis of complex liquid hydrocarbon mixtures such as gasolines. A study of this possibility has resulted in a quick and accurate spectrometric method, which appears to be a worth-while addition to numerous other procedures previously described (*5, 8, 9, 11, 15*).

This method has been applied to some 500 samples, and the resulting data have been used to evaluate laboratory and pilot plant products, commercial grade gasolines, and solvents over a period of 2 years. Analytical data are obtained on total paraffins, total cycloparaffins and/or mono-olefins, aromatics, and the group designated as the "coda" type–namely, cyclomono-olefins, diolefins, and acetylenes.

Calculation of aromatics with unsaturated side chains has not been evaluated here, although their concentration is easily estimated. Normally such compounds are absent but, if present, appear in relatively small and constant amounts. In the case of some catalytically cracked gasolines, for instance, such compounds constitute 6% of all aromatics.

This method reports the calculation of total aromatics only, although average sensitivity data and 10 minutes' additional calculation time are all that is needed to resolve aromatics according to molecular weight.

Mono-olefins are differentiated from cycloparaffins by an auxiliary procedure, such as bromine number or nitrosation (*1*). Accuracy achieved is found to be ±1% for aromatics and ±2% for "coda" compounds in all types of mixtures. Other components are determined within ±2% in wide boiling mixtures but only within ±4% in mixtures of 15° C. or less range. Calibration data presented here can probably be used directly by other

Table I. Numerical Summary of Compounds Studied

No. of Carbon Atoms	Number of Isomers Studied: Aliphatic paraffins	Cyclo-paraffins	Aliphatic mono-olefins	Cyclo-olefins, dienes, acetylenes	Aro-matics
4	2	..	3	2	..
5	3	1	5	3	..
6	5	2	11	2	1
7	9	7	3	2	1
8	18	21	5	2	4
9	10	4	2	..	8
10	1	4	1	..	14
11	0	0	0	..	1
12	1	0	2	..	1
13	1	0	0	..	1
14	1	1	1	..	3
15	0	..	0	..	..
16	2	..	1	..	..
Total	53	40	34	11	34

Total = 172

The determination of compound types in gasoline fractions has been the object of considerable attention by petroleum chemists during the past 25 years. Numerous methods have been developed based on chemical reactions and physical constant data which are, in general, characterized by the need for skilled analysts and rather time-consuming laboratory measurements. In view of the frequently demonstrated ability of instrumental analysis to accomplish what conventional methods cannot, mass spectral data were studied to determine if such data properly used could supplant or supplement other methods of type analysis. Results of the study consist of a mass spectrometric method which is quick and reasonably accurate, and requires only 0.001 ml. of sample. Hydrocarbon types are determined, except for cycloparaffins and monoolefins which are grouped. These latter compounds may then be resolved by chemical analysis, such as nitrosation or bromination. The method can probably be used directly by other mass spectrometer users. In addition, its principle can be extended to obtain type analysis of higher boiling petroleum fractions and mixtures of nonhydrocarbon compounds.

mass spectrometer users as, in general, these data agree well with those published under A.P.I. Research Project No. 44 (see Figure 1). Extension of this method to kerosene and furnace oil fractions has been limited chiefly by the difficulty of obtaining a representative vapor phase sample. It is possible that this could be overcome, however, by the use of a heated inlet system.

METHOD OF ANALYSIS

The applicability of the mass spectrometer to type analysis was determined by a study of mass spectra for the C_4–C_{16} compounds numerically summarized in Table I. The list includes most of the C_4–C_8 paraffins, aromatics through C_9's, and a number of higher molecular weight and olefinic compounds.

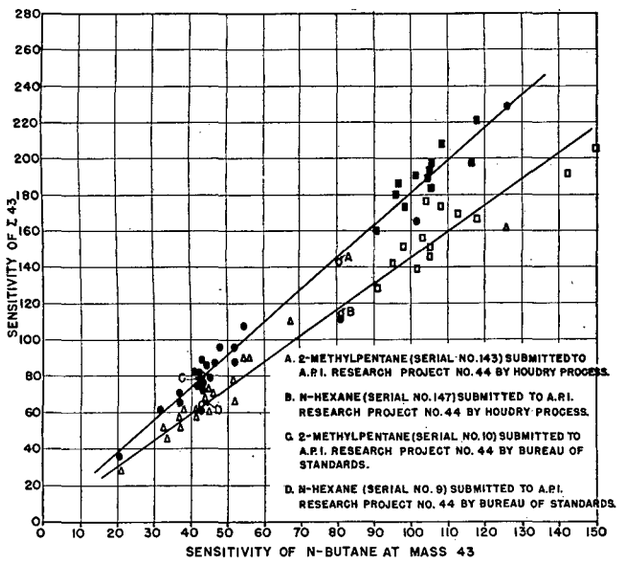

Figure 1. Sensitivity of Σ43 in Mass Spectra of 2-Methylpentane and *n*-Hexane

2-Methylpentane
● **Without temperature control**
■ **With temperature control**
***n*-Hexane**
△ **Without temperature control**
□ **With temperature control**

Comparison of the various spectra showed that ions which appear at m/e^+ ratios 43, 57, 71, 85, and 99 were generally most abundant in paraffins. Similarly, relatively large peaks at 41, 55, 69, 83, and 97 appeared to be characteristic of cycloparaffins and mono-olefins, whereas ions at masses 67, 68, 81, 82, 95, and 96 are contributed generally by cyclo-olefins, diolefins, and acetylenes. Aromatic fragments were found at m/e^+ values of 77, 78, 79, 91, 92, 105, 106, 119, 120, 133, and 134.

When the peaks in each group listed above are mathematically combined and treated as one, it is found that pattern coefficients of combined peaks are similar for compounds of the same type

Table II. Mass Spectra Patterns of Some Aliphatic Paraffins

No. of Carbon Atoms	Compound	Σ41/Σ43 %	Σ53/Σ43 %	Σ67/Σ43 %	Σ77/Σ43 %	Σ69/Σ71 %
4	Isobutane	37.0	..	..	..	..
4	*n*-Butane	29.0	..	..	..	..
	Av.	33.0				
5	Isopentane	46.5	1.5	0.22	0	3.3
5	*n*-Pentane	38.0	1.0	0.09	0	10.0
5	Neopentane	36.7	1.4	0	0	0
	Av.	40.4	1.3	0.16	0	6.7
6	*n*-Hexane	41.5	1.5	0.10	0.04	6.6
6	2-Methylpentane	27.5	1.3	0.16	0.02	2.0
6	3-Methylpentane	51.0	2.5	0.18	0.06	9.0
6	2,2-Dimethylbutane	25.5	1.7	0.14	0.01	0.90
6	2,3-Dimethylbutane	31.8	1.8	0.25	0.03	3.5
	Av.	35.5	1.8	0.17	0.03	4.4
7	*n*-Heptane	31.0	1.3	0.14	0.05	1.7
7	2-Methylhexane	25.0	1.4	0.17	0.08	2.4
7	3-Methylhexane	30.0	1.7	0.24	0.06	2.1
7	3-Ethylpentane	27.5	1.9	0.32	0.06	1.9
7	2,2-Dimethylpentane	24.0	1.4	0.24	0.05	4.7
7	2,3-Dimethylpentane	38.0	2.2	0.33	0.08	5.1
7	2,4-Dimethylpentane	25.0	1.2	0.31	0.05	13.0
7	3,3-Dimethylpentane	18.1	1.7	0.26	0.07	1.5
7	2,2,3-Trimethylbutane	31.0	1.7	0.43	0.07	9.5
	Av.	27.7	1.6	0.27	0.06	4.7
8	*n*-Octane	24.8	1.3	0.23	0.08	2.5
8	2-Methylheptane	25.3	1.4	0.34	0.17	4.7
8	3-Methylheptane	25.5	1.6	0.33	0.13	5.3
8	4-Methylheptane	24.2	1.4	0.37	0.12	3.0
8	3 Ethylhexane	22.5	1.7	0.43	0.14	6.4
8	2,2-Dimethylhexane	23.5	1.5	0.48	0.20	12.5
8	2,3-Dimethylhexane	28.0	1.7	0.62	0.21	3.3
8	2,4-Dimethylhexane	22.7	1.3	0.43	0.14	6.8
8	2,5-Dimethylhexane	23.6	1.4	0.11	0.05	3.8
8	3,3-Dimethylhexane	18.8	1.4	0.48	0.12	4.2
8	3,4-Dimethylhexane	34.0	2.0	0.55	0.26	8.2
8	2,2,3-Trimethylpentane	29.5	1.8	0.59	0.20	24.0
8	2,2,4-Trimethylpentane	25.0	1.3	0.73	0.26	19.0
8	2,3,3-Trimethylpentane	22.0	1.6	0.56	0.14	5.2
8	2,3,4-Trimethylpentane	23.0	1.6	0.52	0.15	2.5
8	2,2,3,3-Tetramethylbutane	24.7	2.4	0.71	0.18	24.3
8	2-Methyl-3-ethylpentane	28.9	1.8	0.63	0.16	7.7
8	3-Methyl-3-ethylpentane	18.5	1.7	0.48	0.08	8.5
	Av.	24.7	1.6	0.48	0.16	8.4
9	*n*-Nonane	24.0	1.4	0.32	0.13	5.0
9	3,3-Diethylpentane	27.0	2.1	1.0	0.20	23.0
9	2,2,3-Trimethylhexane	25.2	1.7	0.66	0.06	14.6
9	2,2,4-Trimethylhexane	20.0	1.5	0.46	0.13	6.0
9	2,2,5-Trimethylhexane	22.0	1.4	0.33	0.16	7.5
9	2,3,3-Trimethylhexane	26.2	1.4	0.63	0.40	17.8
9	3,3,4-Trimethylhexane	27.8	1.8	0.70	0.40	5.7
9	2,2,3,3-Tetramethylpentane	22.0	1.5	0.61	0.15	9.0
9	2,2,3,4-Tetramethylpentane	24.5	1.6	0.67	0.15	18.0
9	2,3,3,4-Tetramethylpentane	26.5	1.4	0.69	0.15	24.5
	Av.	23.7	1.6	0.58	0.15	13.3
10	*n*-Decane	24.2	1.6	0.54	0.08	7.4
10	Hydrogenated triisobutylene	24.3	1.0	0.90	0.19	30.7
16	*n*-Hexadecane	29.8	2.2	2.4	3.0	17.9
12–14	*n*-Paraffins	27.1	1.5	2.4	0.30	15.4
15–17	*n*-Paraffins	26.9	1.5	2.6	0.41	16.1

Table III. Mass Spectra Patterns of Some Cycloparaffins

No. of Carbon Atoms	Compound	Σ43/Σ41 %	Σ53/Σ41 %	Σ67/Σ41 %	Σ77/Σ41 %	Σ71/Σ69 %
5	Cyclopentane	9.2	4.6	3.5	0	148
6	Methylcyclopentane	14.8	6.7	6.0	0.60	3.6
6	Cyclohexane	18.8	9.5	4.4	1.1	17.8
	Av.	16.8	7.0	5.2	0.90	10.7
7	Methylcyclohexane	5.7	4.9	12.3	0.70	4.1
7	Ethylcyclopentane	7.8	5.8	36.7	0.60	4.1
7	1,1-Dimethylcyclopentane	11.2	4.3	5.0	0.60	6.6
7	*cis*-1,2-Dimethylcyclopentane	15.1	7.7	4.3	1.1	15.6
7	*trans*-1,2-Dimethylcyclopentane	16.4	7.8	5.1	1.0	12.5
7	*cis*-1,3-Dimethylcyclopentane	14.4	6.4	4.8	0.90	9.9
7	*trans*-1,3-Dimethylcyclopentane	15.8	6.6	5.1	1.1	12.1
	Av.	12.3	6.2	10.5	0.90	9.3
8	1,1-Dimethylcyclohexane	16.4	4.9	10.1	1.2	0.60
8	*cis*-1,2-Dimethylcyclohexane	12.7	5.6	10.8	1.1	3.8
8	*trans*-1,2-Dimethylcyclohexane	15.0	5.7	10.9	1.1	2.9
8	*cis*-1,3-Dimethylcyclohexane	12.9	4.9	10.3	1.5	2.5
8	*trans*-1,3-Dimethylcyclohexane	9.7	5.3	10.2	2.3	2.1
8	*cis*-1,4-Dimethylcyclohexane	11.4	5.9	12.3	1.4	1.8
8	*trans*-1,4-Dimethylcyclohexane	10.9	5.4	10.5	1.2	1.7
8	1-Methyl-1-ethyl cyclopentane	5.6	3.6	16.5	0.80	3.1
8	*cis*-1-Methyl-2-ethyl cyclopentane	12.5	5.5	13.3	1.1	6.4
8	*trans*-1-Methyl-2-ethyl cyclopentane	12.8	5.0	14.9	1.0	6.8
8	*cis*-1-Methyl-3-ethyl cyclopentane	13.9	4.3	20.5	0.90	7.4
8	*trans*-1-Methyl-3-ethyl cyclopentane	11.9	4.3	20.0	0.90	5.6
8	Isopropylcyclopentane	19.5	4.2	58.9	1.0	1.1
8	*n*-Propylcyclopentane	14.4	5.6	34.2	0.90	4.4
8	1,1,2-Trimethylcyclopentane	24.3	5.1	5.5	1.2	3.7
8	1,1,3-Trimethylcyclopentane	26.2	4.3	6.6	1.6	0.90
8	*cis,cis,cis*-1,2,3, Trimethylcyclopentane	20.0	6.4	3.8	0.80	49.3
8	*cis,cis,trans*-1,2,3-Trimethylcyclopentane	22.4	5.8	4.0	1.3	40.5
8	*cis,trans,cis*-1,2,3-Trimethylcyclopentane	24.0	6.1	4.9	1.3	22.5
8	*cis,cis,trans*-1,2,4-Trimethylcyclopentane	16.2	5.6	5.5	1.3	30.5
8	*cis,trans,cis*-1,2,4-Trimethylcyclopentane	15.8	5.4	5.4	1.2	21.3
	Av.	15.6	5.2	13.8	1.2	10.4
9	Isobutylcyclopentane	23.0	4.9	33.2	1.3	2.1
9	Isopropylcyclohexane	8.2	6.5	40.1	1.3	0.40
9	*n*-Propylcyclohexane	5.6	5.7	34.8	1.0	0.90
9	1,1,3-Trimethylcyclohexane	13.7	3.9	6.9	1.6	2.4
10	*trans*-Butylcyclohexane	94.7	9.8	34.4	2.5	6.7
10	*sec*-Butylcyclohexane	9.1	6.6	45.0	1.6	0.80
10	Isobutylcyclohexane	13.7	5.5	32.5	1.4	0.80
10	*n*-Butylcyclohexane	7.1	5.5	37.0	1.3	1.2
14	*p*-Di-*tert*-butylcyclohexane	153.0	6.0	35.2	4.5	12.4

but show marked differences for dissimilar compounds. Furthermore, sensitivities of these peaks are found to be directly related to molecular weight. These properties make it possible, then, to consider complex mixtures as consisting of only four components which may be resolved with four simultaneous equations.

Similarity of pattern coefficients for compounds of the same type is demonstrated in Tables II to VI, inclusive. Paraffin coefficients in Table II are based on either Σ43 or Σ71, where Σ43 denotes the sum of peaks at masses 43, 57, 71, and 85 and Σ71 the sum of 71, 85, and 99. (Other sigma values shown in the tables are defined in Table VII.) These patterns are essentially the same, although a slight shift occurs with an increase in the number of carbon atoms in a molecule, and this same observation applies to other compound types listed in Tables III to VI. To compensate for the pattern difference which exists between molecular weight groups, average coefficients have been calculated corresponding with C_6 to C_9 hydrocarbons as shown in Table VII. These are values estimated on the basis that mixtures of a given molecular weight consist of molecules, approximately 80% of which correspond to the measured molecular weight and 20% are divided between the next lower and higher homologs. Thus C_8 coefficients are weighted as 10% C_7's, 80% C_8's, and 10% C_9's. This scheme has been found adequate for both narrow and wide boiling mixtures, although it is less suitable for the latter type of mixture. Coefficients are essentially independent of filament and operating conditions; only in the case of Σ41/Σ43 paraffin patterns was any effect noticed. Here a 7% change occurred when the magnet current was changed from 0.56 to 0.70 ampere.

Table IV. Mass Spectra Patterns of Some Mono-olefins

No. of Carbon Atoms	Compound	Σ43/Σ41 %	Σ53/Σ41 %	Σ67/Σ41 %	Σ77/Σ41 %	Σ71/Σ69 %
4	Isobutene	1.9	6.2	0	0	0
4	1-Butene	1.6	6.5	0	0	0
4	2-Butene	1.9	9.2	0	0	0
	Av.	1.8	7.3	0	0	0
5	1-Pentene	5.0	5.8	1.5	0	100
5	2-Pentene	3.3	7.8	1.6	0	91.5
5	2-Methyl-1-butene	3.3	8.4	1.4	0	68.0
5	2-Methyl-2-butene	3.9	9.2	2.6	0	80.0
5	3-Methyl-1-butene	4.4	8.8	0.80	0	63.0
	Av.	4.0	8.0	1.6	0	80.5
6	1-Hexene	37.0	7.6	1.2	0.38	11.5
6	*trans*-2-Hexene	9.2	9.5	2.0	0.44	0.40
6	*trans*-3-Hexene	9.8	7.8	2.4	0.39	8.1
6	2,3-Dimethyl-1-butene	5.4	5.3	3.3	0.39	2.1
6	3,3-Dimethyl-1-butene	6.4	4.7	1.5	0.08	1.5
6	2-Methyl-1-pentene	9.4	7.2	2.4	0.33	5.8
6	2-Methyl-2-pentene	9.4	4.7	3.2	0.40	5.8
6	3-Methyl-1-pentene	4.8	8.2	1.6	0.16	2.8
6	*cis*-3-Methyl-2-pentene	5.2	8.0	4.0	0.40	3.4
6	4-Methyl-1-pentene	108[a]	5.0	3.1	0.60	5.6
6	*cis*-4-Methyl-2-pentene	4.8	4.6	3.6	0.34	2.1
	Av.	10.7	6.6	2.4	0.32	4.6
7	1-Heptene	24.0	8.4	3.8	0.36	10.5
7	4,4-Dimethyl-1-pentene	113[a]	3.7	2.9	0.57	2.8
7	2,3,3-Trimethyl-1-butene	11.7	3.0	2.8	0.44	2.1
	Av.	17.9	5.0	3.2	0.46	5.1
8	1-Octene	58.0	7.6	6.1	0.43	18.0
8	2-Octene	29.6	8.3	7.6	0.90	18.9
8	*trans*-4-Octene	11.8	6.6	5.8	1.2	8.3
8	2,4,4-Trimethyl-1-pentene	155	6.2	3.5	1.7	0.50
8	2,4,4-Trimethyl-2-pentene	25.0	7.1	6.4	4.3	0.15
9	1-Nonene	66.0	8.4	8.0	0.54	14.3
9	Nonenes	25.0	4.9	7.5	2.4	11.5
10	1-Decene	62.2	8.7	11.2	1.0	15.8
12	1-Dodecene	65.7	7.9	14.8	0.30	24.2
12	Triisobutylene	240	6.5	13.4	4.7	2.5
14	1-Tetradecene	72.3	7.3	18.3	2.0	26.1
16	1-Hexadecene	82.1	12.5 (estd.)	17.0	13.7	31.7

[a] Not included in average.

Analysis of a sample requires accurate sensitivity in addition to pattern coefficient data. Because it is not feasible to run a large number of calibrations with each unknown sample it appeared necessary to relate sensitivities with a standard such as *n*-butane. A study of this possibility showed combined peak sensitivities to be directly related to that of *n*-butane at mass 43 irrespective of filament and operating conditions. Figure 1, for instance, shows the sensitivity of Σ43 for *n*-hexane and 2-methylpentane observed for twelve different filaments. Similar plots for as many compounds as possible were used to obtain the average curves in Figures 2 to 8, inclusive, which show that sensitivities vary directly with molecular weight. These curves and daily *n*-butane sensitivities are used in computing mixtures.

Table V. Mass Spectra Patterns of Some Aromatics

No. of Carbon Atoms	Compound	Σ41/Σ77 %	Σ43/Σ77 %	Σ53/Σ77 %	Σ67/Σ77 %	Σ69/Σ77 %	Σ71/Σ77 %
6	Benzene	0.04	0	0.70	0	0	0
7	Toluene	1.18	1.4	0.70	0.06	0.01	0.34
8	1,2-Dimethylbenzene	1.17	0.19	1.9	0.05	0	0.08
8	1,3-Dimethylbenzene	1.19	0.18	2.0	0.04	0	0.09
8	1,4-Dimethylbenzene	1.02	0.22	2.2	0.04	0	0.09
8	Ethylbenzene	1.0	0.19	0.86	0.03	0	0.06
9	1,2,3-Trimethylbenzene	2.2	0.50	2.2	0.20	0.09	0.05
9	1,2,4-Trimethylbenzene	2.3	0.40	2.2	0.20	0.07	0.04
9	1,3,5-Trimethylbenzene	2.2	0.30	2.2	0.20	0.04	0.03
9	1-Methyl-2-ethylbenzene	1.3	0.30	1.5	0.08	0.02	0.01
9	1-Methyl-3-ethylbenzene	1.4	0.30	1.6	0.08	0.03	0.03
9	1-Methyl-4-ethylbenzene	1.3	0.30	1.5	0.08	0.04	0.03
9	Isopropylbenzene	2.2	0.30	1.0	0.04	0.03	0.02
9	*n*-Propylbenzene	1.6	0.12	0.47	0.07	0.07	0.02
10	1,2-Dimethyl-3-ethylbenzene	3.7	0.50	1.9	0.30	0.10	0.10
10	1,2-Dimethyl-4-ethylbenzene	3.5	0.50	1.9	0.40	0.10	0.10
10	1,3-Dimethyl-2-ethylbenzene	3.4	0.50	1.8	0.30	0.09	0.10
10	1,3-Dimethyl-4-ethylbenzene	3.4	0.40	1.8	0.30	0.08	0.09
10	1,3-Dimethyl-5-ethylbenzene	3.5	0.40	2.0	0.40	0.10	0.10
10	1,4-Dimethyl-2-ethylbenzene	3.5	0.50	1.9	0.30	0.20	0.10
10	1,2-Diethylbenzene	3.0	0.47	1.3	0.29	0.26	0.06
10	1,3-Diethylbenzene	3.0	0.45	1.3	0.28	0.55	0.09
10	1,4-Diethylbenzene	2.7	0.35	1.2	0.25	0.21	0.07
10	1-Methyl-4-isopropylbenzene	5.3	0.92	1.4	0.27	0.13	0.14
10	*n*-Butylbenzene	2.5	0.28	0.51	0.04	0.01	0.01
10	Isobutylbenzene	5.1	7.3	0.49	0.10	0.02	0.02
10	*sec*-Butylbenzene	2.2	0.10	0.73	0.05	0.04	0.02
10	*tert*-Butylbenzene	10.0	0.75	0.80	0.08	0.24	0.16
14	1,2-Diisopropylbenzene	8.0	3.5	1.2	0.27	0.35	0.20
14	1,3-Diisopropylbenzene	6.7	11.2	1.1	0.15	0.15	0.19
14	1,4-Diisopropylbenzene	6.3	8.1	1.1	0.22	0.13	0.27

C_4–C_8 paraffins and aromatics (Figures 2 to 5, 8) are based on numerous calibration standards and appear to be representative. C_9's and olefin sensitivities, on the other hand, are considered adequate but less reliable, owing to the lack of pure compounds, particularly the coda type.

A Consolidated mass spectrometer Model No. 21-101, equipped with temperature control and deflector plates, was used with the sample introduction system modified by replacing stopcocks with mercury-sealed sintered disks (*13*) and Teflon cutoff valves. This modification minimized hydrocarbon sorption in the inlet system and made possible the analysis of normally liquid hydrocarbons found in the gasoline boiling range.

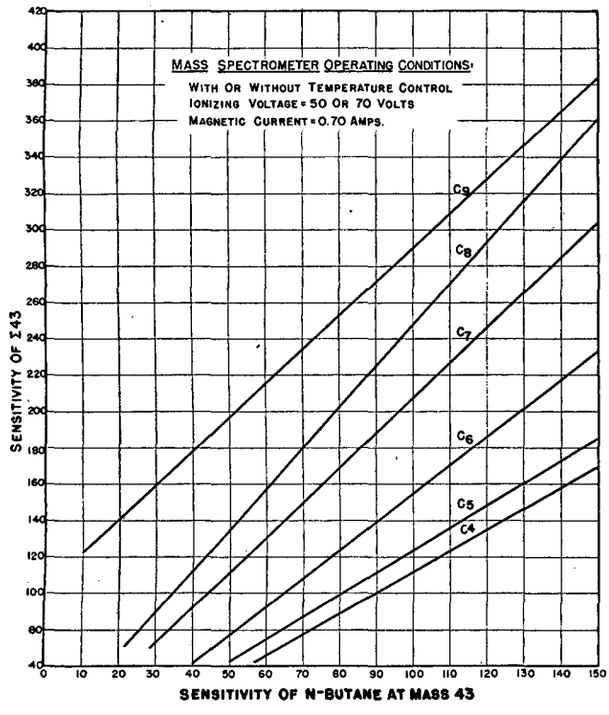

Figure 2. Sensitivity of Σ43 in Mass Spectra of Aliphatic Paraffins

PROCEDURE AND CALCULATION

A sample to be analyzed is first stripped of C_5 and lighter compounds in a low temperature distillation column to minimize sampling errors, which occur on introduction of liquids containing volatile compounds to the mass spectrometer. The distillation bottoms are introduced to the spectrometer inlet system, using the microburet technique of Taylor and Young (*13*). The observed sample pressure and density are then used to calculate an average molecular weight which serves as a guide in selecting pattern coefficients from Table VII, and a bromine number is obtained to indicate the concentration of olefins.

Coefficients selected from Table VII are used to set up four simultaneous equations to calculate total paraffins, total cycloparaffins and/or monoolefins, aromatics, and the coda group. The solution to the simultaneous equations based on combined peaks Σ43, Σ41, Σ67, and Σ77, gives peak heights from which partial pressures may be calculated using sensitivities obtained from Figures 2 to 8. A check calculation may be obtained by

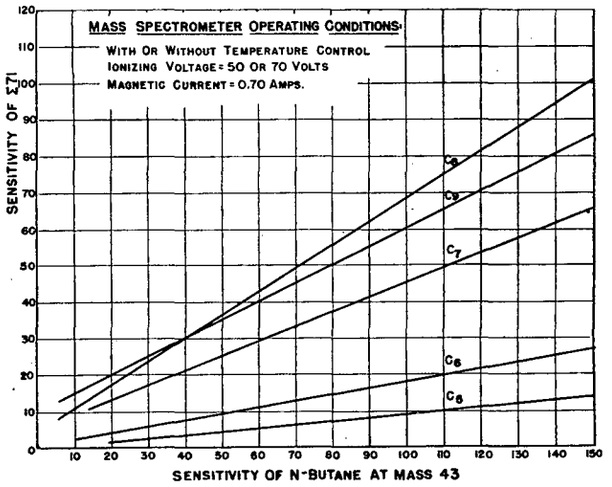

Figure 3. Sensitivity of Σ71 in Mass Spectra of Aliphatic Paraffins

Table VI. Mass Spectra of Some Cyclo-olefins, Diolefins, and Acetylenes

No. of Carbon Atoms	Compound	Σ41/Σ67 %	Σ43/Σ67 %	Σ69/Σ67 %	Σ71/Σ67 %	Σ77/Σ67 %
6	Cyclohexene	35.4	1.1	2.5	0.2	11.5
7	3-Methyl-1-cyclohexene	30.0	0.5	2.3	0.0	8.8
5	2-Methyl-1,3-butadiene	23.6	0.8	2.7	0.0	0.0
6	1,5-Hexadiene	115	0.7	0.2	0.0	4.6
8	2,5-Dimethylhexadiene	101	5.5	6.9	0.3	6.9
6	1-Hexyne	62.2	45.4	0.3	0.0	3.7
7	1-Heptyne	86.4	24.6	2.1	1.6	5.1
8	1-Octyne	80.4	55.1	13.2	4.0	6.3

subtracting contributions of other types at Σ53 and considering the residual peak as due to coda compounds. Two simultaneous equations based on Σ69 and Σ71 are used for a check of paraffins and the cycloparaffin–mono-olefin group. Normally average values from the two independent calculations are used to obtain the final analysis. Further details of the calculation are illustrated at the end of the discussion.

ACCURACY OF METHOD

Data presented in Tables VIII, IX, and X show results obtained for various samples. A synthetic sample containing seventy-six C_6–C_{12} hydrocarbons and blended so as to have an average molecular weight corresponding to a C_8 was analyzed to within 0.2 to 1.0 of the correct volume per cent, as shown in Table VIII.

The original sample and the distillation bottoms of a gasoline were analyzed on the mass spectrometer before and after removal of olefins by nitrosation, and the olefins were determined by difference (Table IX). The olefin content of C_6 and C_7 fractions 1 to 3, however, is based on mass spectrometer analysis of silica gel fractions, because the difference method noted above is not applicable to narrow boiling fractions (Table X, Sources of Error). Probable accuracy of the method is indicated by close agreement between the mass spectrometer olefin determinations and those by bromine number and silica gel percolation. Comparison of the direct analysis for the composite sample with that calculated from the composition of the various fractions shows

Table VII. Mass Spectra Patterns of Hydrocarbons

(Mass Spectrometer Operating Conditions. With or without temperature control. Ionizing voltage = 50 or 70 volts. Magnet current = 0.70 ampere)

No. of Carbon Atoms in Molecule	Σ41/Σ43[a]	Σ53/Σ43	Σ67/Σ43	Σ77/Σ43	Σ69/Σ71
	Aliphatic Paraffins				
	%	%	%	%	%
6.0	38.0	1.7	0.17	0.03	4.4
6.5	34.2	1.6	0.23	0.05	4.8
7.0	30.3	1.6	0.29	0.07	5.1
7.5	28.4	1.6	0.38	0.11	6.8
8.0	26.6	1.6	0.47	0.15	8.5
8.5	26.1	1.6	0.52	0.15	10.8
9.0	25.6	1.6	0.58	0.15	13.0
	Cycloparaffins				
	Σ43/Σ41	Σ53/Σ41	Σ67/Σ41	Σ77/Σ41	Σ71/Σ69
6.0	16.8	6.7	5.2	1.1	10.2
6.5	15.5	6.4	7.8	1.0	9.9
7.0	14.2	6.2	10.3	0.9	9.6
7.5	14.6	5.8	12.9	1.1	9.6
8.0	15.0	5.4	15.3	1.2	9.6
8.5	15.0	5.3	24.3	1.3	9.6
9.0	15.1	5.2	32.9	1.3	9.6
	Mono-olefins				
	Σ43/Σ41	Σ53/Σ41	Σ67/Σ41	Σ77/Σ41	Σ71/Σ69
6.0	10.7	6.6	2.4	0.32	4.6
6.5	15.0	6.0	2.9	0.46	5.1
7.0	19.2	5.4	3.4	0.59	5.5
7.5	28.1	6.3	4.7	1.12	7.5
8.0	37.0	7.1	5.8	1.66	9.4
8.5	43.5	7.2	7.4	1.46	11.4
9.0	50.0	7.3	8.9	1.25	13.4
	Cyclo-olefins, Diolefins, Acetylenes				
	Σ41/Σ67	Σ43/Σ67	Σ69/Σ67	Σ71/Σ67	Σ77/E67
6.0	32	10	1.0	0.0	4
6.5	34	10	1.3	1	5
7.0	35	10	1.5	1	5
7.5	55	12	14	2	7
8.0	75	13	25	2	8
8.5	..	..	..	..	..
9.0	..	..	..	..	..
	Aromatics				
	Σ41/Σ77	Σ43/Σ77	Σ53/Σ77	Σ67/Σ77	Σ69/Σ77	Σ71/Σ77
6.0	0.04	0.0	0.7	0.0	0.0	0.0
6.5	0.6	0.7	0.7	0.03	0.0	0.17
7.0	1.2	1.4	0.7	0.06	0.01	0.34
7.5	1.2	0.7	1.2	0.05	0.0	0.21
8.0	1.2	0.4	1.7	0.04	0.0	0.08
8.5	1.6	0.3	1.6	0.07	0.03	0.05
9.0	2.0	0.3	1.5	0.10	0.05	0.03

Sum of Peaks	Masses
Σ41	41, 55, 69, and 83
Σ43	43, 57, 71, and 85
Σ53	53 and 54
Σ67	67, 68, 81, 82, 95, and 96
Σ69	69, 83, and 97
Σ71	71, 85, and 99
Σ77	77, 78, 79, 91, 92, 105, 106, 119, 120, 133, 134, etc.

[a] For mixtures run at a magnet current of 0.56 ampere, Σ41/Σ43 patterns are corrected by a factor of 0.934.

Table VIII. Analysis of Mixture Containing 76 C_6–C_{12} Hydrocarbons

Hydrocarbon Type	Volume %, Known		Volume %, M.S.		Diff.
Aliphatic paraffins	36.3		37.2		0.9
Cycloparaffins	23.4	44.0	43.0		−1.0
Mono-olefins	20.6				
Cyclo-olefins	0.0				
Diolefins	0.0		0.4		0.4
Acetylenes	0.0				
Aromatics	19.7		19.4		−0.3
Total vol. %	100.0		100.0		

Table IX. Analysis of a Gasoline

(Volume per cent)

	Gasoline	Fraction 1 (C_6 Cut)	Fraction 2 (C_7 Cut)	Fraction 3 (C_7 Cut)	Bottoms	Composite Analysis (Based on Compn. of Various Fractions)
Aliphatic paraffins	43.4	58.5	45.0	42.3	36.7	46.9
Cycloparaffins	19.8	8.3	28.4	29.2	23.8	21.1
Mono-olefins	17.0	28.5	15.9	10.6	12.5	16.2
Cyclo-olefins, Diolefins, Acetylenes	6.6	2.7	9.1	7.9	6.2	5.0
Aromatics	13.2	2.0	1.6	10.0	20.8	10.8
	100.0	100.0	100.0	100.0	100.0	100.0
% Olefins (total)						
M.S.	23.6	31.2	25.0	18.5	18.7	..
Bromine No.	23.1	31.2	22.1	17.5	15.8	..
Silica gel	..	28.4	23.5	22.2	..	..

Olefins in original and bottoms samples were obtained by difference as determined by mass spectrometer analysis of samples with and without olefins (olefins removed by nitrosation). Composition of fractions 1 to 3 is based on mass spectrometer analyses of silica gel cuts of each sample.

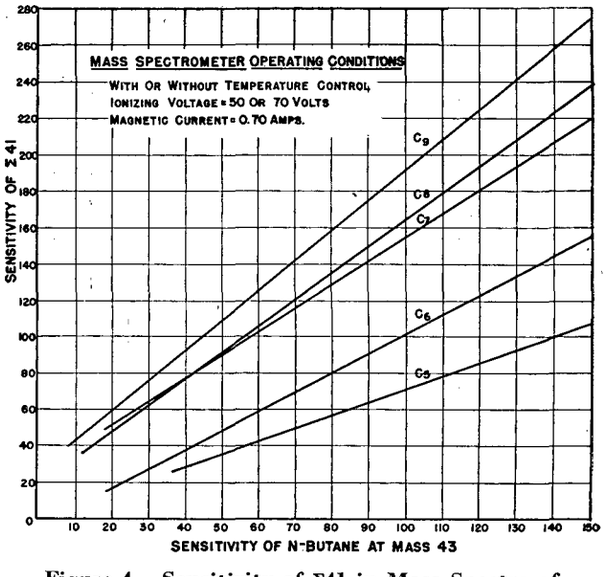

Figure 4. Sensitivity of Σ41 in Mass Spectra of Cycloparaffins

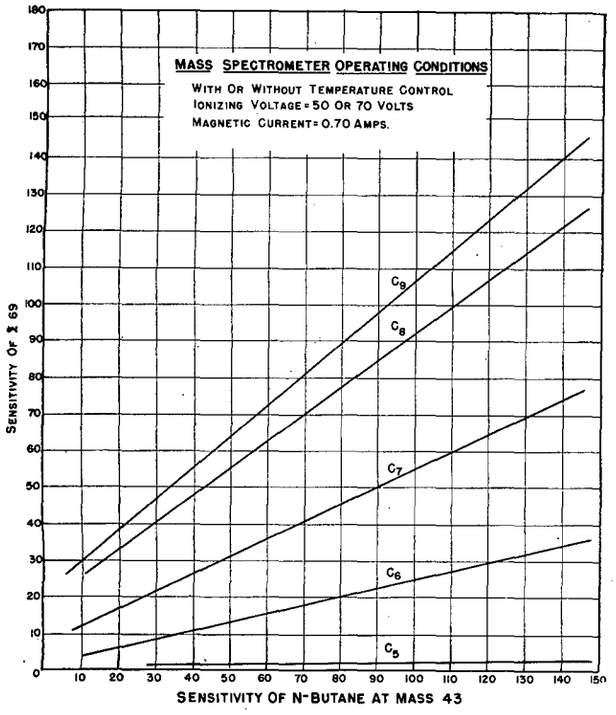

Figure 5. Sensitivity of Σ69 in Mass Spectra of Cycloparaffins

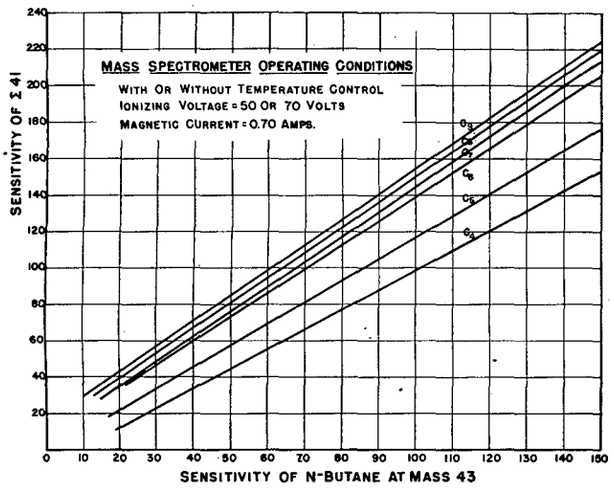

Figure 6. Sensitivity of Σ41 in Mass Spectra of Mono-olefins

fairly good agreement. The rather large discrepancy in the aromatic values is probably a sampling error and not due to the method of calculation, because reliable results are generally obtained for this compound type.

Analysis of several narrow boiling paraffin-aromatic mixtures is shown in Table X along with the "known" composition, which actually is that based on an accurate determination of all the compounds in the mixture. Such analyses, in general, agree within ±4% in the case of paraffins and ±0.5% for aromatics, although paraffins appear systematically low in the compound-type method. These samples, however, were calculated some time before the present method of analysis was devised and such systematic errors are believed to be no longer present.

Table X. Analysis of Close Boiling Point Mixtures

Mixture No.	Av. No. Carbon Atoms	Aliphatic Paraffins		Cycloparaffins		Aromatics	
		Known[a]	M.S.	Known[a]	M.S.	Known[a]	M.S.
1	6	82.2	77.8	8.8	12.5	9.0	9.7
2	6	85.5	81.9	9.8	13.4	4.7	4.7
3	6	81.6	75.6	13.9	19.8	4.6	4.6
4	6	72.9	69.6	26.9	30.2	0.2	0.2
5	6	2.2	3.4	94.1	93.7	3.7	2.9
6	6.5	73.6	69.9	24.4	28.1	2.0	2.0
7	7	100	98.5	0.0	1.5	0.0	0.0
8	7	77.4	75.0	22.6	24.9	0.0	0.1
9	7	61.5	68.6	38.5	31.4	0.0	0.0
10	7	65.2	66.5	23.2	22.5	11.6	11.0
11	7.5	45.1	44.5	16.8	17.0	37.9	38.5
12	7.5	7.2	1.4	92.8	98.6	0.0	0.0
13	7.5	23.8	20.5	14.5	15.8	61.7	63.7
14	7.5	45.9	43.9	36.1	37.4	18.0	18.7
15	7.5	51.1	54.3	43.8	40.6	5.1	5.1
16	8	13.7	9.2	86.3	90.8	0.0	0.0
17	8	65.9	65.8	34.1	34.2	0.0	0.0
18	8	31.4	26.1	68.6	73.9	0.0	0.0
19	8	63.7	62.8	36.3	37.1	0.0	0.1
20	8	68.3	75.6	31.7	24.4	0.0	0.0

[a] Known composition determined by complete resolution of compounds in mixture.

SOURCES OF ERROR

Deviation in the sensitivities and pattern coefficients of compounds with the same molecular weight is a major source of error. Such errors are most marked in the case of narrow boiling mixtures, because, otherwise, cancellation of errors takes place due to the presence of a large number of compounds. Data in Table XI of sensitivity deviations for C_4-C_8 paraffins show that the sensitivities of most compounds are of the same order of magnitude, although exceptions to this rule occur at Σ71 for *n*-hexane, 2,4-dimethylpentane, 3-methylpentane, 2,2,3-trimethylpentane, and 2,2,4-trimethylpentane and at Σ69 for the propyl and trimethylcyclopentanes. Pattern coefficients are essentially constant except for 4-methyl-1-pentene, 2,4,4-trimethyl-1-pentene (Table IV), coda compounds in general, and the Σ67/Σ41 patterns observed for cycloparaffins (Table III). Because this last pattern is vital for an accurate analysis of coda compounds, the presence of cycloparaffins reduces the accuracy of this determination.

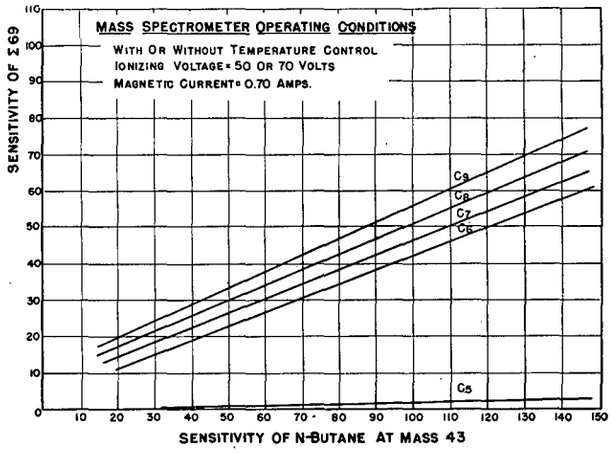

Figure 7. Sensitivity of Σ69 in Mass Spectra of Mono-olefins

A serious source of error results from the lack of calibration compounds of the olefinic type, particularly the cyclomono-olefins, for which sensitivity data are the least reliable of all compounds. Determination of these compounds is undoubtedly the most inaccurate part of the analysis on a relative basis, although the error is reduced somewhat by use of the check calculation provided.

Table XI. Deviation of Sensitivity for C_4–C_8 Paraffins

Compound	Maximum % Deviation of Av. Sensitivity Curve from Pure Compound Σ43	Σ71
Isobutane	..	..
n-Butane	..	..
Isopentane	−5	..
n-Pentane	6	..
n-Hexane	6	187
2-Methylpentane	−15	−53
3-Methylpentane	14	305
2,2-Dimethylbutane	−25	−69
2,3-Dimethylbutane	−1	−22
n-Heptane	15	−6
2-Methylhexane	−4	−13
3-Methylhexane	12	−14
3-Ethylpentane	6	−31
2,2-Dimethylpentane	−22	−3
2,3-Dimethylpentane	26	42
2,4-Dimethylpentane	−11	110
3,3-Dimethylpentane	−22	−60
2,2,3-Trimethylbutane	−2	20
n-Octane	−5	−19
2-Methylheptane	5	63
3-Methylheptane	−1	−3
4-Methylheptane	−2	−30
2,3-Dimethylhexane	9	−13
2,4-Dimethylhexane	−14	−16
2,5-Dimethylhexane	3	44
3,3-Dimethylhexane	−18	−49
3,4-Dimethylhexane	24	40
2,2,3-Trimethylpentane	15	500
2,2,4-Trimethylpentane	−2	500
2,3,3-Trimethylpentane	−5	−34
2,3,4-Trimethylpentane	−10	−34
	Σ41	Σ69
Cyclopentane	..	..
Methylcyclopentane	−10	−22
Cyclohexane	11	40
Methylcyclohexane	−13	−38
Ethylcyclopentane	20	−14
1,1-Dimethylcyclopentane	−23	−47
cis-1,2-Dimethylcyclopentane	20	83
trans-1,2-Dimethylcyclopentane	14	66
cis-1,3-Dimethylcyclopentane	20	41
trans-1,3-Dimethylcyclopentane	20	55
1,1-Dimethylcyclohexane	2	−34
cis-1,2-Dimethylcyclohexane	5	−24
trans-1,2-Dimethylcyclohexane		
cis-1,3-Dimethylcyclohexane	15	−24
trans-1,3-Dimethylcyclohexane		
cis-1,4-Dimethylcyclohexane	6	
trans-1,4-Dimethylcyclohexane		
1-Methyl-1-ethylcyclopentane	−31	−29
cis-1-Methyl-2-ethylcyclopentane	−16	33
trans-1-Methyl-2-ethylcyclopentane		
cis-1-Methyl-3-ethylcyclopentane		
trans-1-Methyl-3-ethylcyclopentane		
Isopropylcyclopentane	32	154
n-Propylcyclopentane		
1,1,2-Trimethylcyclopentane	−9	11
1,1,3-Trimethylcyclopentane	−6	−18
cis,cis,cis-1,2,3-Trimethylcyclopentane	16	166
cis,cis,trans-1,2,3-Trimethylcyclopentane		
cis,trans,cis-1,2,3-Trimethylcyclopentane		
cis,cis,trans-1,2,4-Trimethylcyclopentane	12	
cis,trans,cis-1,2,4-Trimethylcyclopentane		

The presence of such compounds in catalytically cracked gasoline has been definitely established, however, by analyses such as reported in Table IX.

Resolution of mono-olefins and cycloparaffins is based on bromine number or nitrosation and is consequently subject to the limitations of these methods. According to a recent article by Dinneen *et al.* (*4*) nitrosation is more reliable than bromination, as olefins determined by the latter method may be systematically high. Because mono-olefins are calculated as the difference between olefins determined chemically and the coda compounds of the mass spectrometer analysis, validity of the chemical determination is of prime importance. Of lesser concern than the mono-olefin value itself is the effect that this determination has on the selection of pattern coefficients to be used in calculations

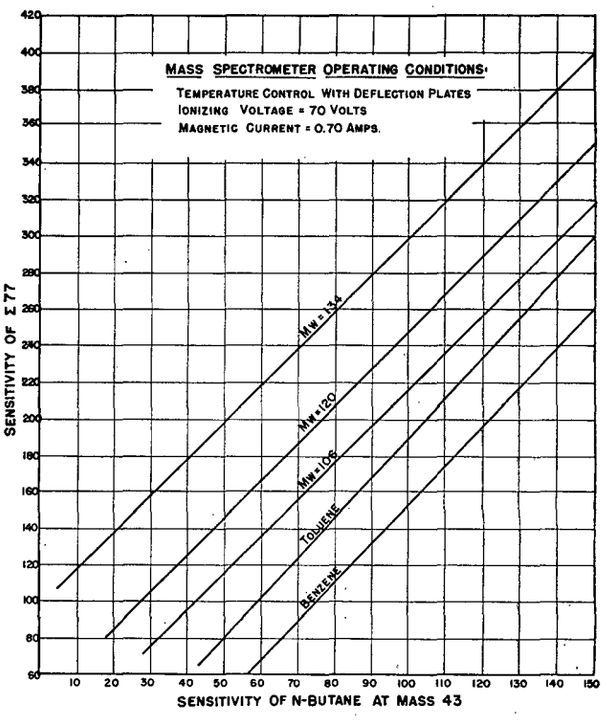

Figure 8. Sensitivity of Σ77 in Mass Spectra of Aromatics with Saturated Side Chains

(see calculations 3*b* and 3*c*), as these vary somewhat with the ratio of cycloparaffin to mono-olefin.

CALCULATIONS

1. A gasoline sample of 57.3-micron pressure was scanned from mass 32 to 186 at a magnet current of 0.70 ampere. Peaks in the mass spectra necessary for the calculation were:

m/e^+	Peak Height	m/e^+	Peak Height
41	1920	91	607
43	2349	92	179
53	176	95	3.8
54	102	96	14.0
55	1356	97	215
57	1491	99	65.5
67	111	105	602
68	104	106	193
69	577	119	81.4
71	572	120	182
77	136	133	0.6
78	171	134	56.4
79	79	..	..
81	19.9	147	0
82	172	148	0
83	560		
85	403		

2. Calculation of Σ values (figures in parentheses represent m/e^+ values).

$$\Sigma 41 = \overset{(41)}{1920} + \overset{(55)}{1356} + \overset{(69)}{577} + \overset{(83)}{560} = 4413$$

Similarly,

Σ43 = 4815	Σ67 = 425	Σ71 = 1041
Σ53 = 278	Σ69 = 1352	Σ77 = 2287

3*a*. A molecular weight determination based on the microburet technique (*13*, *17*) indicated that eight carbon atoms per molecule should be used in calculations.

b. A bromine number indicated approximately 25% olefin material. The selection of pattern coefficients for the cycloparaffin–mono-olefin group was based on a 50-50 distribution of these two types of compounds. (Had the bromine number indicated 0% olefins, for instance, coefficients corresponding to cycloparaffins only would have been used.)

c. Using the coefficients shown in Table VII and the mass spectra of the mixture, four simultaneous equations were set as follows:

$$0.266\ P + 1.00\ O\&C.P. + 0.75\ \text{Coda} + 0.012\ A = 4413$$
$$1.00\ P + 0.26\ O\&C.P. + 0.13\ \text{Coda} + 0.004\ A = 4815$$

$$0.0047\,P + 0.105\,O\&C.P. + 1.00\ \text{Coda} + 0.0004\,A = 425$$
$$0.0015\,P + 0.014\,O\&C.P. + 0.08\ \text{Coda} + 1.00\,A = 2287$$

On a routine basis precalculated inverse solutions are used to solve such equations. These inverses reduce the analysis time and also enable a calculator to employ various combinations of coefficients for a single sample. This latter procedure is followed whenever a partial calculation indicates that a cycloparaffin–mono-olefin distribution other than that being used is applicable. Choice of pattern coefficients is limited to five cycloparaffin–mono-olefin ratios—namely, 0, 0.33, 1.0, 3.0, and ∞.

Solution		Sensitivity[a]	Partial Pressure, μ
Aliphatic paraffins	= 3942	190 (Figure 2)	20.8
Mono-olefins and/or cycloparaffins	= 3293	121 (Figures 4,6)	27.2
Coda	= 59	150[b]	0.4
Aromatics	= 2230	176[c] (Figure 8)	12.7

[a] Based on *n*-butane sensitivity of 74.5 at mass 43.
[b] Sensitivity of Σ67 coda value is estimated by equation: sensitivity = 0.85 × sensitivity Σ77 of aromatics. This equation represents an approximation of sensitivities observed for compounds in Table VI. It has no known theoretical significance and was selected for convenience only.
[c] The sensitivity of Σ77 for the aromatics is determined graphically by using a value for the average number of carbon atoms which is 0.4 unit higher than the average determined. In this case, therefore, the sensitivity of 176 was that obtained for 8.0 + 0.4 or 8.4 carbon atoms per molecule. Basis for this procedure lies in the empirical observation that the mean molecular weights of aromatics in a number of gasoline blends were consistently higher than the rest of the sample. In many cases, however, this correction may not be justified and should be checked when applied to a number of samples from the same source.

4. Check Calculations.

a. The paraffins and mono-olefins are further checked by means of two simultaneous equations based on Σ69 and Σ71. To do this, contributions of other types must be removed—for example:

$$\Sigma 69 = 1352,\ \Delta(\Sigma 69) = 1352 - 59 \times 0.25 - 2230 \times 0.00 = 1337$$
$$\Sigma 71 = 1041,\ \Delta(\Sigma 71) = 1041 - 59 \times 0.002 - 2230 \times 0.0008 = 1039$$

Equations.

$$0.085\,P + 1.00\,O\&C.P. = 1337$$
$$1.00\,P + 0.095\,O\&C.P. = 1039$$

Solution		Sensitivity	Partial Pressure, μ
Paraffins	= 921	52.5 (Figure 3)	17.6
Olefins – cycloparaffins	= 1259	56.8 (Figure 5)	22.2

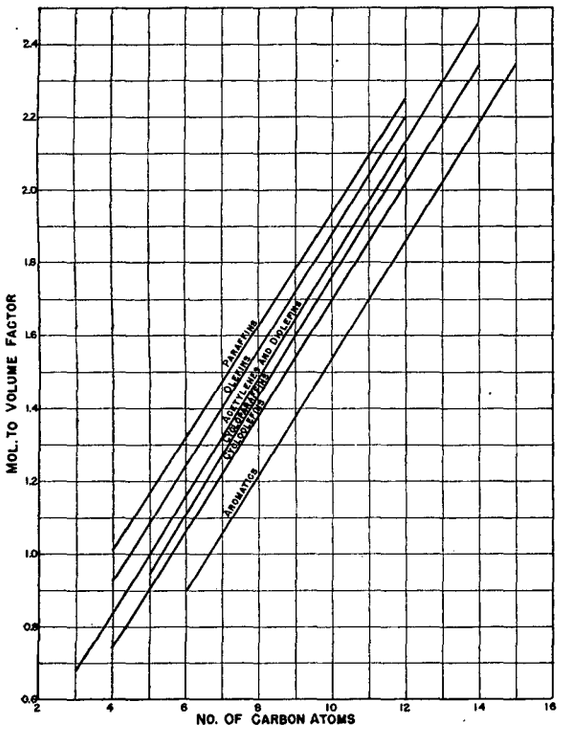

Figure 9. Mol. to Volume Factors for C_4 to C_{12} Hydrocarbons

b. The coda type compounds are next checked by calculating a residual Σ53:

$$\Sigma 53 = 278,\ \Delta(\Sigma 53) = 278 - 3942 \times 0.016 - 3293 \times 0.063 - 2230 \times 0.017 = -30$$

The negative residual indicates the absence of coda type compounds. Had a positive answer been obtained, the residual peak would have been converted to a partial pressure, using a sensitivity value 37.8, which is estimated by the equation,

$$\text{Sensitivity of } \Sigma 53 = 0.4\,(\text{sensitivity of } n\text{-butane at mass } 43) + 8$$

5. Normally, samples are reported in terms of volume per cent. The final calculation would therefore be outlined:

	Aliphatic Paraffins	Cycloparaffins and/or Mono-olefins	Coda	Aromatics
Partial pressure				
Method 1	20.8	27.2	0.4	12.7
Method 2	17.6	22.2	0.0	...
Mean	19.2	24.7	0.2	12.7
Mole to volume factor (Figure 9)	1.63	1.47	1.43	1.28
Volume	31.3	36.3	0.3	16.3Σ = 84.2
Volume %	37.2	43.0	0.4	19.4Σ = 100.0

CONCLUSION

Gasoline distillates and petroleum solvents may be analyzed according to compound type on a Consolidated Model 21-101 mass spectrometer equipped with a modified inlet system. Constituent compounds, such as aliphatic paraffins; cycloparaffins and/or mono-olefins; cyclo-olefins, diolefins, and/or acetylenes; and aromatics are determined within ±4%. Mono-olefins are determined by bromine number or nitrosation. Three man-hours are required for a complete analysis and it is believed the data presented here can be used directly by other users of mass spectrometers.

ACKNOWLEDGMENT

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Analysis of Solids with the Mass Spectrometer

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Although the mass spectrometer has been strikingly successful in recent years in the analysis of mixtures of gases and vapors, there has been no parallel success in the routine analysis of solids. This is because the most versatile source, the high frequency spark, has been too erratic for the conventional mass spectrometric method. The aim in the present work was to develop a method whereby the spark source could be used in an instrument employing electrical recording. A method of compensating for fluctuations in the source is illustrated in the determination of the nickel and chromium content of six stainless steels. The results agree nicely with the composition determined chemically. This initial success makes it evident that the mass spectrometer should find wide application in the immediate future in the routine, rapid analysis of solids.

THE striking success attained with the mass spectrometer in recent years in analyzing complex gas mixtures has quickened interest in the possible application to the analysis of solids.

Dempster (*1, 3*) has illustrated the determination of traces with his precision mass spectrograph, which had been developed for the measurement of packing fractions. However, the photographic method has inherent limitations in accuracy, convenience, speed, linearity, and range of concentrations that may be covered on one exposure. In addition, the sensitivity of the photographic plate decreases for heavier masses. Some of these limitations are discussed by Dempster, and it is possibly these limitations that led him to conclude that this application will be slow in developing, and to stress the comparison with optical spectroscopy where "fifty years elapsed before optical spectrographs were applied to any extent in industry." This comparison is also stressed by Inghram (*5*). A mass spectrograph for analyzing solids was constructed by Shaw and Rall (*7*), using the conventional photographic plate as detector, but apparently little application has been made to analysis, possibly because of the limitations just mentioned.

Many of these limitations are removed by the use of electrical in place of photographic detection, as in the mass spectrometer for gases. A mass spectrometer of the conventional type requires a "quiet source" which produces an ion beam of very constant intensity. For some very specialized problems in analyzing solids, such sources are available. Hickam (*4*) has described a method of determining impurities in copper by completely vaporizing the sample in a furnace closely adjacent to the ionization region and integrating the ion current at selected masses until the sample is exhausted. This method, although sensitive, is very limited in application.

The best available source for solids is generally accepted to be the vacuum spark source (*5, 7*). Its very erratic nature has previously limited its application almost entirely to photographic detection—i.e., the mass spectrograph rather than the mass spectrometer. In this paper is described a successful method of using the spark source with the mass spectrometer, in which the accuracy attained with chemically analyzed samples of stainless steels is comparable with that for gases and vapors on the conventional instrument. The results compare favorably with the best that has been done with the optical spectrograph.

PRINCIPLE OF THE METHOD

After it has been decided that the most generally useful method would be the use of the spark source with electrical detection, the problem is to devise some means for compensating for the wild fluctuations in the current from the spark. The use of the "isotope-ratio" circuit of Nier, Ney, and Inghram (*6*) suggests itself. In their application, the effect of the source fluctuations was minimized and the precision of the determination of the ratio of two isotopes was increased by simultaneously focusing and measuring the ions corresponding to the two isotopes on separate collectors; their electronic circuit then measured the ratio of these two currents rather than the intensity of each separately. Thus, the accuracy obtainable with the gas-type source was improved. This principle was then employed in the Consolidated-Nier instrument, which was designed to measure isotopic ratios in gases and vapors.

The isctope-ratio method must be revised in the application to general analysis, because a complete spectrum is required and it is not feasible to have a separate collector for each mass. It is most convenient for this work to have a single exit slit and ion collector and sweep the various successive masses across this exit slit. In the authors' work, a monitoring collector placed at the entrance to the magnetic analyzer provides a measure of the ions of all masses that enter the analyzer. The magnetic field is then varied and the ions of various masses are scanned across the exit slit of the magnetic analyzer. The currents are amplified and the

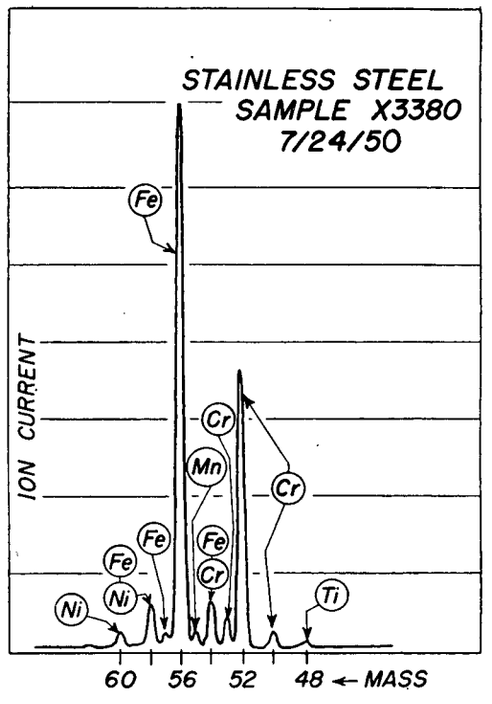

Figure 1. Mass Spectrum of Stainless Steel Sample Obtained from Spark Source
Retrace of original pen-and-ink recording

Table I. Calculation of Ionization Factors Using Sample X3534

Element	Mass Used	Relative Ion Current	Relative Ion Current Corrected for: Overlapping isotope	Relative Ion Current Corrected for: Isotope abundance	Relative Concentration in Beam	Determined Chemically	Ionization Factor
Chromium	52	56.6	56.6	62.0	57.8	38.5	1.50
Iron	56	100.0	100.0	100.0	100.0	100.0	1.00
Nickel	58	14.2	13.9	18.7	19.7	22.1	0.89

Table II. Determination of Composition of Sample X3522

Element	Mass Used	Relative Ion Current[a]	Relative Ion Current Corrected for: Overlapping isotope	Relative Ion Current Corrected for: Isotope abundance	Relative Concentration: In beam	Relative Concentration: In sample	Composition, %
Chromium	52	6.3 ± 0.1	6.3	6.9	6.4	4.2	2.97 ± 0.05
Iron	56	100.0 ± 0.1	100.0	100.0	100.0	100.0	70.1 ± 0.1
Nickel	58	23.3 ± 0.3	23.0	31.0	32.6	36.6	25.6 ± 0.3
Other	..	.....	...	...	...	...	1.33

[a] Mean value and average deviation of 5 runs.

pen-and-ink recorder records the ratios of the various types of ions emerging from the analyzer to the current measured by the monitoring electrode. Because the monitoring collector receives an ion current composed of all masses, and the final ion collector receives the current corresponding to only one mass at a time, the two collectors will receive currents that tend to fluctuate in the same manner—i.e., in the manner in which the source is fluctuating.

The appearance of the spectrum obtained in this way is illustrated in Figure 1. This sample is a stainless steel and the major components are immediately evident. The record has the same appearance as that from the gas analyzer. In fact, the same compensating method used here could also be applied to the mass spectrometers for analyzing mixtures of gases and vapors and for measuring isotopic ratios.

THE INSTRUMENT

The instrument will be described in greater detail in another article and the analytical method is emphasized here.

A mass spectrograph of the Dempster type (*2*) was available for this work. In this instrument, the ions undergo a deflection of 90° in an electrostatic field and are then bent 180° in a magnetic field. The electrostatic deflection is necessary because of the wide distribution in energy of the ions emerging from the spark source. The electrostatic deflectors allow only those ions in a narrow energy band to enter the magnetic analyzer; this is necessary for proper focusing at the exit slit. The basic form of this instrument was retained, but considerable modifications were made to the various components.

The electrostatic deflection was changed to 45° in order that an electron multiplier could be placed at the exit slit; in the older arrangement the region around the ion receiver was crowded by the proximity of the ion source. The electron multiplier has not yet been used, although it is ready for mounting on the unit. This will be valuable in the study of trace components, but was not necessary in demonstrating the practicability of this analytical method. A sliding vacuum valve was inserted between the ion source and the rest of the instrument, so that the spark electrodes could be changed without losing vacuum in the entire system. A new housing was constructed for the region at which the ions approach the magnet to permit the proper mounting and positioning of the monitoring electrode. The analyzer housing was revised to allow for the insertion of a rotating fluxmeter into the magnet gap in order that the ions reaching the final collector could be identified according to their masses. The assembly in the region of the ion receiver was modified so that a slit and ion collector could be substituted for the photographic plate.

The source is the one described by Shaw and Rall (*7*). In place of the Tesla coil, the spark is powered by an interrupted oscillator substituted several years ago by R. H. Britten. This oscillator operates at about 1 megacycle and is turned on for an interval of 200 microseconds 60 times per second. The spark takes place between a rod and a disk. In the authors' work, the sample was machined to the form of a rod with a diameter of 0.025 inch, while the disk was made from tantalum. Each time the sample was changed, a new clean disk was used to avoid contamination from the previous sample. Experience has shown that the analysis is not critically dependent on the position of the rod relative to the disk, and for analytical work it would usually be more convenient to spark between two rods of the same material. The average monitor current obtained from this source was about 5×10^{-11} ampere, and the average ion current at the final collector for the largest peak was about 5×10^{-12} ampere. The recorder system showed adequate precision for the larger peaks, but because of its limited amplification, the reproducibility of the smaller peaks was poor. For this reason, a galvanometer and manual ratio potentiometer were used for the analyses reported here. The addition of an amplifier to the recorder should permit automatic recording with the desired precision on all peaks.

ANALYTICAL METHOD

As in optical spectroscopy, an internal standard is required—i.e., the absolute amount of an individual component is not determined but only its concentration relative to other components present. For convenience, the authors have referred everything to iron in the following presentation.

The first step is to calibrate the instrument by means of a sample whose composition is known. Possibly the clearest explanation of the analytical procedure would be provided by tracing through this procedure with an illustrative example. This is done with the aid of Table I for the relative concentration of iron, nickel, and chromium. The trace components are not determined in this presentation, the purpose of which is to demonstrate the effectiveness of this new technique. The measurement of the trace elements appears to be a simple extension of the principles presented here. The means of doing this are at hand, but the curtailment of this project in the immediate future makes it desirable to make the present incomplete results generally available.

In making this analysis, mass 52 was used for chromium, mass 56 for iron, and mass 58 for nickel. In column 3 of Table I, the relative ion currents at these masses are indicated. It is known that iron has an isotope at mass 58 which is 0.3% of 56. This correction to the mass 58 peak is made and the relative peak height due to Ni^{58} is shown in column 4. In column 5, the data are adjusted to account for the fact that chromium and iron each have four stable isotopes and nickel has five. Although mass 56 is used as a measure of the amount of iron present, the iron present at other mass numbers must also be considered. From tables of isotopic abundance, it is determined that Fe^{56} accounts for only 91.6% of the iron present in the sample, the rest being Fe^{54}, Fe^{57}, and Fe^{58}. Similarly, Cr^{52} accounts for only 83.7% of the chromium in the sample, the rest being Cr^{50}, Cr^{53}, and Cr^{54}. Therefore, the figure 56.6 in column 4 for chromium becomes (100/83.7) 56.6, and for iron (100/91.6)100. Nickel is similarly adjusted and these three revised values are normalized to Fe = 100 and entered in column 5. This column now gives the relative numbers of atoms of chromium, iron, and nickel in the ion beam received at the ion collector. Using the values of the atomic weights, the relative concentration by weight in the beam is obtained in column 6. This is not necessarily the same as the distribution of components in the sample, because the efficiency of creating ions in the source varies with the different elements in the sample. Likewise, the ions may be sorted preferentially by the analyzer. The factor by which the relative concentration of a component in the sample must be multiplied to give the corresponding relative concentration in the beam is called the ionization factor. These factors are here determined by comparison with the composition of the sample determined chemically. The chemical determinations are shown in column 7. The ionization factors given in column 8 are obtained by dividing the figures in column 6 by the corresponding ones in column 7.

Table III. Summary of Analyses of Stainless Steel Samples

Sample	Chromium		Nickel		Iron		Other Elements
	Chemical, %	M.S., %[a]	Chemical, %	M.S., %[a]	Chemical, %	M.S., %[a]	Chemical, %
X3534	23.7	23.4 ± 0.4	13.6	13.7 ± 0.5	61.6	61.8 ± 0.4	1.1
X3380	18.2	19.3 ± 0.3	8.6	8.7 ± 0.2	69.8	68.6 ± 0.3	3.4
X3275	13.4	13.7 ± 0.2	0.37	0.25 ± 0.01	83.0	82.8 ± 0.3	3.2
X3532	9.1	9.1 ± 0.2	0.57	0.56 ± 0.02	85.5	85.5 ± 0.3	4.8
X3533	5.5	5.5 ± 0.1	6.8	7.1 ± 0.1	84.3	84.0 ± 0.1	3.4
X3522	2.95	2.97 ± 0.05	25.7	25.6 ± 0.3	70.0	70.1 ± 0.1	1.3

[a] Mean value and average deviation of 4 or 5 runs.

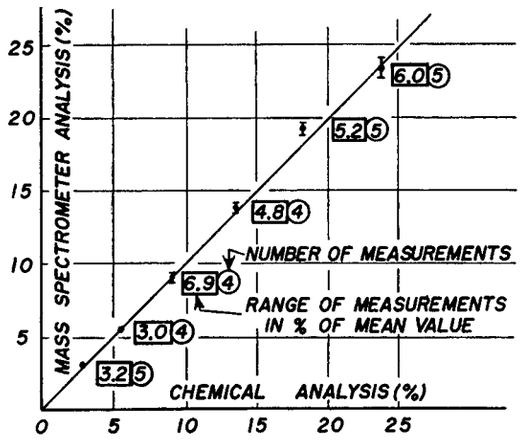

Figure 2. Comparison with Chemical Determination of Chromium

The ionization factors that have been determined may now be used to analyze a sample. This is illustrated for sample X3522 in Table II. The first six columns are the exact parallel of the corresponding ones in Table I. However, the ionization factors listed in Table I may now be used to obtain the relative concentration of chromium, iron, and nickel in the sample (column 7) from the measured concentration in the beam (column 6). The desired analysis for X3522 has now been attained. For convenient comparison with the chemical determination, the result is expressed in percentage in column 8. The chemical analysis of 1.33% for the other components is assumed to be correct. This assumption is necessary only because the development of the mass spectrometric method was incomplete at the writing of this manuscript.

For routine analyses, the steps outlined above can be shortened. Once the correction for overlapping isotope is made to the relative ion current (Table I), an over-all calibration factor for each element can be obtained by dividing the ion current by the relative concentration determined chemically. Then in Table II, the relative concentration in the sample (column 7) would be obtained directly by dividing the figure of column 4 by this calibration factor.

Six stainless steel samples covering convenient concentration ranges were made available for this work by the bureau's chemistry division. The samples had been prepared and the chemical compositions determined by the Uddeholms Aktiebolag in Hagfors, Sweden. If sample X3534 is used for calibration, the other five can be analyzed. Actually, the ionization factors were chosen so that a good fit on all the samples was obtained, still assuming the calibration to be linear with concentration. This makes a very slight change in the calibration obtained with X3534 (Table I). The only ionization factor affected is chromium which becomes 1.53 instead of 1.50.

The data are summarized in Table III and compared with the chemical determination. To illustrate the reproducibility of the mass spectroscopic results, the average deviations from the mean of four or five determinations in each case are shown. The sample was not removed from the instrument between these runs, but on several other runs the sample was removed between runs with no significant change in the analysis. Sample X3380 was checked in this way because of its deviation from the chemical determination for chromium. This check run gave 18.9% chromium, as contrasted with 19.3% in Table III. Furthermore, this check run was made with both rod and disk in the spark source fabricated from sample X3380. In all previous runs, the disk was made of tantalum and the rod was formed from the sample.

The data of Table III are plotted in Figures 2 and 3 to illustrate the linearity of the calibration curve and the nice check in most cases with the chemical determination. Perfect agreement between the mass spectrometric and the chemical methods would be indicated by the measurement falling on the 45° line in these figures. The vertical line at each point indicates the range in which the measurements fell.

CONCLUSION

The results are better than it had been anticipated would be possible with a spark source. The interpretation is very easy because of the simplicity of the spectrum. The results that have been presented were taken over a period of 5 weeks, and there was no apparent shift in calibration during this period. The linearity as regards concentration is an attractive feature which eases the calibration problem. The changing of the sample is time-consuming in the present arrangement, but with an arrangement designed specially for analytical work it would be possible to interchange samples in a few minutes.

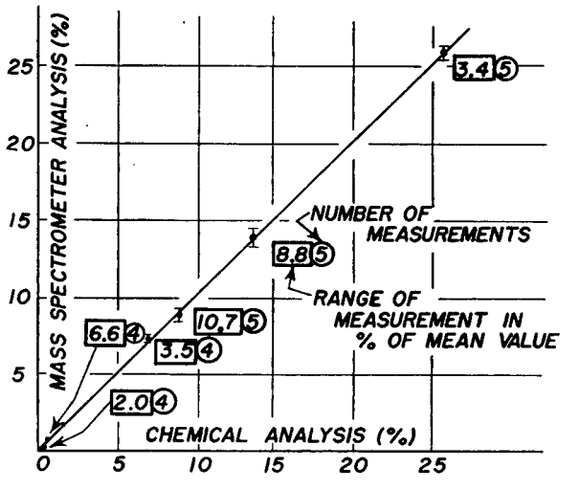

Figure 3. Comparison with Chemical Determination of Nickel

Although the method has been illustrated here with metallic samples, for which it is particularly well suited, nonmetallic samples have been studied photographically with the spark source in several mass spectrographs. These studies indicate that analyses with the present method should be feasible, although possibly less accurate. The successful results on the stainless steel samples indicate that the mass spectrometer should have a wide field of application in the quantitative analysis of solids.

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Spectrophotometric Determination of Total Phthalic Anhydride

In Alkyd Resins and Other Phthalate Esters

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The standard Kappelmeier procedure for the determination of total phthalic anhydride in alkyd resins and other phthalate esters cannot be applied in the presence of esters of other polycarboxylic acids, many unsaponifiable resins, and other materials used in the formulation of modern surface coatings. The work described was undertaken with a view to fulfilling the widespread need for a more specific and generally applicable method for this type of determination. A new method based on ultraviolet absorption spectrophotometry and possessing a much wider range of application than the Kappelmeier method was developed. Accurate results are rapidly obtained from a single absorption measurement at 276 mμ on an acidified aqueous solution of the usual Kappelmeier precipitate. The method should prove especially useful in laboratories concerned with the analysis of surface coatings and related materials.

THE well known Kappelmeier method (*3*) and its various modifications have for many years represented the most satisfactory means for estimating the total phthalic acid (or anhydride) content of alkyd resins and other phthalate esters. Unfortunately, however, this method fails completely in the presence of any material that is insoluble in anhydrous alcoholic alkali or that yields a saponification product insoluble in this medium. Because such materials include monomeric and polymeric esters of practically all other polycarboxylic acids, unsaponifiable or partially saponifiable resins, pigments, etc., this limitation represents a serious handicap in the analysis of paints, varnishes, and other types of coating materials. The need for a more specific method for phthalic as well as generally improved techniques in the analysis of present-day coating materials has been emphasized in several recent publications (*2, 5, 6*).

When the Kappelmeier precipitate contains only potassium phthalate and completely water-insoluble material, the phthalic content can sometimes be estimated by weighing the dried residue after aqueous extraction of the precipitate. It is also possible in some cases to obtain an approximate estimate by heating the precipitate containing the phthalic as the monoalcoholate to constant weight at elevated temperature and assuming the weight loss to be due to volatilization of the alcohol of crystallization. These procedures, however, have obvious limitations and fall far short of filling the need for a more specific and reliable method for the phthalic determination.

Swann in a recent publication (*6*) has described methods for determining phthalic, sebacic, fumaric, maleic, succinic, and adipic acids in alkyd resins. His procedure for phthalic acid is the only published method claimed to be directly applicable in the presence of other dibasic acids. The method requires preliminary isolation of the dibasic acids as dry solids by acidification and evaporation of the dissolved Kappelmeier precipitate; phthalic acid is then precipitated as the lead salt from glacial acetic acid. While this method is useful, it is time-consuming and suffers from the disadvantages inherent in procedures involving precipitation of lead salts. By comparison, the ultraviolet spectrophotometric method is much more rapid and generally satisfactory. Swann's methods for the other dibasic acids mentioned above probably represent the best now available, although more recent studies (*4*) suggest the application of partition chromatography to this general problem.

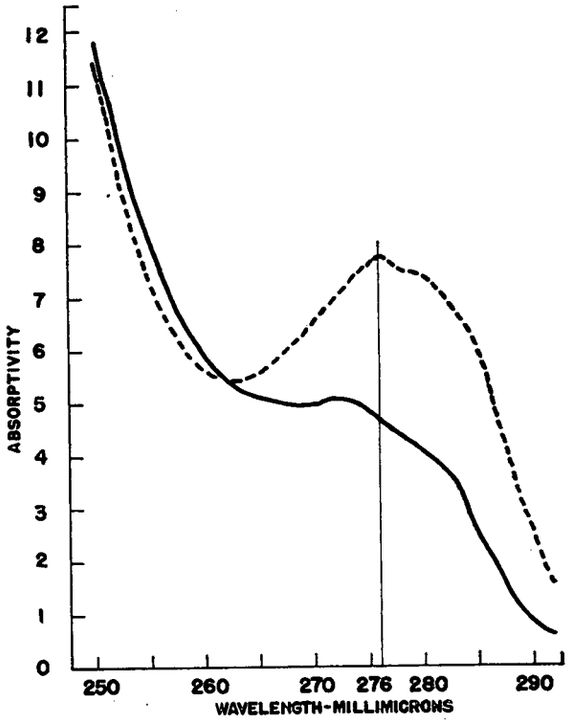

Figure 1. Ultraviolet Absorption Spectrum of Potassium Phthalate Monoalcoholate

— In distilled water, phthalate ion 0.0469 gram per liter
--- In aqueous 0.1 *N* hydrochloric acid, phthalic acid 0.0500 gram per liter

In the present investigation the ultraviolet spectrophotometric method has been shown to yield excellent results in determination of the phthalic content of alkyd resins and other phthalate esters, alone or in the presence of a variety of esters of other polybasic acids, including those studied by Swann (*6*). It has also been successfully applied to this type of determination in the presence of other coating materials that interfere with the Kappelmeier procedure.

Table I. Absorptivity of Potassium Acid Phthalate in 0.1 *N* Hydrochloric Acid at 276 mμ

Concn., G./L. Phthalic Acid	Absorptivity
0.0246	7.84
0.0344	7.76
0.0492	7.73
0.0640	7.74
0.0836	7.76

BASIS OF SPECTROPHOTOMETRIC METHOD

Phthalic acid and its esters show strong absorption in the ultraviolet region of the spectrum, this absorption arising from electronic transitions accompanying resonance in the aromatic ring present. In some types of mixtures it should be possible to determine the phthalate ester content by direct absorption measurements on a solution of the mixture in a suitable ultraviolet transparent solvent. In the case of paint vehicles and other types of coating materials, however, such a procedure would be impractical, because the organic solvents, oils, and other materials usually present would almost invariably interfere. Fortunately, however, application of the Kappelmeier saponification procedure followed by aqueous extraction of the precipitate obtained offers a simple and effective means for completely eliminating this difficulty in most cases and greatly minimizing the probability of interference in all cases. This is true because relatively few of those materials likely to appear in the Kappelmeier precipitate possess the simultaneous properties of water solubility and strong ultraviolet absorption under the conditions selected for the phthalic acid determination.

Figure 1 shows the ultraviolet absorption spectrum of aqueous potassium phthalate monoalcoholate (distilled water being used in the reference cell) and that of the same compound in 0.1 *N* hydrochloric acid, with 0.1 *N* hydrochloric acid in the reference cell. "Absorptivity" in Figure 1 is equivalent to specific extinction coefficient in the older nomenclature—i.e., absorptivity

$$= \frac{\log I_0/I}{\text{Concn. in grams per liter} \times \text{cell length}}.$$

In the case of the neutral salt, concentration of phthalate ion was used in calculating the absorptivity values; in the case of the acidified salt, concentration of phthalic acid was used. Because the inherent absorption of the free acid (acidified salt) is greater than that of the phthalate ion (neutral salt), a method based on the absorption of the acidified salt was adopted. Exact acid concentration is not critical in the analytical procedure but should be held constant at about 0.1 *N*. Although phthalic acid shows stronger absorption at shorter wave lengths, the wave length of the absorption maximum at 276 mμ was selected for the analysis for the following reasons:

Unsaturated dibasic acids such as maleic, fumaric, and itaconic show strong absorption at shorter wave lengths. Even the saturated dibasic acids, as commercially obtained, may show some absorption at shorter wave lengths but negligible absorption in the region selected.

In general, the probability of interference from small amounts of unsaturated water-soluble organic materials which might be extracted with the phthalate salt is greater at shorter wave lengths.

The absorptivity of phthalic acid at 276 mμ (7.73 under the conditions employed) is sufficient to ensure accuracy and precision comparable with those of the Kappelmeier method, not only in the case of straight phthalate esters but in the presence of large amounts of a number of materials that interfere with the latter method.

The adherence of phthalic acid (as acidified potassium acid phthalate) to Beer's law is demonstrated by the data of Table I.

MATERIALS AND APPARATUS

Spectrophotometer. All absorption measurements were made using a Beckman Model DU quartz ultraviolet spectrophotometer, housed in a constant temperature room maintained at 25° C. Matched fused silica absorption cells 1 cm. in length were used.

Calibration Standards. Baker's C.P. potassium acid phthalate and Eastman *o*-phthalic acid (purified by recrystallization) gave identical results in the calibration procedure described below (a_p = 7.73 under the conditions employed).

Polycarboxylic Acids and Esters. The polycarboxylic acids used in preparing the synthetic acid mixtures were the purest commercial grades obtainable. With the exception of phthalic acid, they were used without further purification.

The alkyd resin used was an oil-modified glyceryl phthalate type especially prepared for this investigation. The monomeric phthalate esters and dibutyl maleate were the purest obtainable commercial grades. The adipate and sebacate polyesters were commercially available plasticizing resins. The various monomeric saturated esters were pure samples kindly supplied by H. B. Knight of the Eastern Regional Research Laboratory. The polystyrene and polyvinyl chloride-acetate samples were commercial grades and the styrenated alkyd was synthesized for the purpose.

ANALYTICAL PROCEDURE

Calibration. Calibration consists of determining the absorptivity (specific extinction coefficient) of pure phthalic acid in approximately 0.1 *N* hydrochloric acid at 276 mμ on the spectrophotometer. Because potassium acid phthalate is obtainable in a high state of purity, the acidified solution of this salt is a convenient standard.

Weigh accurately about 0.12 gram of pure potassium acid phthalate into a calibrated 1-liter volumetric flask, dissolve, add 10 ml. of concentrated hydrochloric acid, and dilute to the mark with distilled water. Measure 50 ml. of this solution from a buret into a 100-ml. volumetric flask and dilute to the mark with approximately 0.1 *N* hydrochloric acid (prepared by diluting 10 ml. of concentrated hydrochloric acid to 1 liter with distilled water). Measure the absorbance (optical density) at 276 mμ against 0.1 *N* hydrochloric acid (10 ml. of concentrated hydrochloric acid per liter) as blank, using matched 1-cm. silica or quartz absorption cells. Repeat the measurement with the hydrochloric acid solution in the cell previously used for the sample, and sample solution in the cell previously used as blank. Average the measurements to obtain the absorbance, A, and calculate the absorptivity of phthalic acid, a_p, from the formula:

$$a_p = \frac{A}{C_p} \qquad (1)$$

where C_p is concentration expressed as grams per liter of phthalic acid (not salt) present in the final diluted solution.

One calibration suffices for all subsequent analyses unless conditions are changed.

Procedure for Analysis of Samples. Accurately weigh into suitable reflux flasks three samples of the paint vehicle or other phthalate-containing material, using a sample size expected to yield the equivalent of about 0.1 gram of phthalic acid on saponification. Add 5 ml. of benzene and 35 ml. of 1 *N* absolute alcoholic potassium hydroxide to each flask, attach to a reflux condenser, and reflux 1.5 hours.

From this point on follow the standard A.S.T.M. procedure (*1*), or other approved procedure, for isolating and drying the precipitate, which will contain the phthalic as dipotassium phthalate or the monoalcoholate (depending on the drying procedure used), together with any other materials present which are insoluble in the saponification medium. Unless the salt of an interfering acid is present (see below), the precipitate need not be weighed.

Extract the soluble salts thoroughly from one of the three precipitates by passing about 200 ml. of distilled water through the crucible with suction. To the aqueous extract in the suction flask, add 10 ml. of concentrated hydrochloric acid. If sebacic acid or other slightly soluble acidic material is present, a precipitate or cloudiness may appear at this point. If the acidified solution is perfectly clear, transfer with rinsing to a 1-liter volumetric flask and dilute to the mark with distilled water; if, however, even a slight cloudiness is evident in the acidified extract in the suction flask, filter the entire solution with suction through No. 42 Whatman filter paper on a Büchner funnel, wash the residue with about 100 ml. of distilled water, transfer the combined filtrate and washings to the 1-liter volumetric flask, and dilute to the mark with distilled water. After thorough mixing, introduce from a buret a 50-ml. aliquot of the diluted extract into

a 100-ml. volumetric flask and dilute to the mark with 0.1 N hydrochloric acid (10 ml. of concentrated hydrochloric acid per liter). Measure the absorbance at 276 mμ against a 0.1 N hydrochloric acid blank, using the technique specified under calibration above.

For maximum accuracy, the absorbance of the final solution measured should fall in the vicinity of 0.4 absorbance unit.

If the absorbance lies between 0.35 and 0.45, dissolve the other two Kappelmeier precipitates and proceed exactly as in the case of the first. Calculate the phthalic acid concentration in grams per liter, C_p, of each of the final diluted solutions from the formula:

$$C_p = \frac{A}{a_p} \qquad (2)$$

where A is the measured absorbance and a_p is the absorptivity value for pure phthalic acid as determined in the calibration procedure above.

Calculate the per cent of total phthalic anhydride in the solid portion of the original sample from the following formula:

$$\% \text{ P.A.} = \frac{89.16\, C_p \times \text{aliquot factor}}{W \times S} \qquad (3)$$

where W is sample weight and S is fractional solids content of the original sample if a volatile solvent was present.

Average the three values obtained.

If the absorbance of the final diluted extract from the first sample lies outside the 0.35 to 0.45 range, calculate the approximate phthalic acid concentration from Equation 2. With this knowledge at hand, extract the other two precipitates (filtering after acidification if necessary), and adjust the volume to which the extract is diluted or the aliquot taken for final dilution or both, so as to obtain absorbance values in the desired range. In order to minimize dilution error, the volume of the original solution in each case should be such that not more than a fourfold final dilution is required.

If a mixture is being analyzed, the per cent phthalic anhydride calculated from Formula 3 can, of course, be converted to equivalent phthalate ester content by applying the appropriate factor.

Use of Alcohol for Final Dilution. When filtration of the acidified extract is necessary, small amounts of insoluble matter may pass through the filter in some cases. An apparently clear acidified extract may sometimes contain very small amounts of suspended matter not readily detected. The presence of such material will cause error in the absorbance measurement due to scattering of the ultraviolet radiation. The difficulty can usually be eliminated by using 0.1 N hydrochloric acid in absolute synthetic methanol, or a spectroscopic grade of ethyl alcohol, in the final dilution of the aqueous aliquot taken. In fact, users of the method may wish to adopt the use of alcohol at this point as constant standard practice, in order to minimize the possibility of scattered radiation error. When alcohol is used, the potassium biphthalate solution and blank used for calibration as well as the blank used in the sample measurement must be adjusted to the solvent composition present in the sample solution.

Presence of Interfering Acids. The method cannot be directly applied in the presence of acids showing strong ultraviolet absorption at 276 mμ under the conditions of the analysis. However, in the presence of one interfering acid, which exhibits only moderate absorption at this wave length, such as maleic or fumaric, accurate results can be obtained as follows:

Determine the absorptivitiy, a_i, of a pure sample of the interfering acid (suitably diluted in 0.1 N hydrochloric acid) at 276 mμ; then apply the method exactly as outlined above, but calculate C_p from the following pair of simultaneous equations rather than from Equation 2:

$$a_p C_p + a_i C_i = A \qquad (4)$$

$$F_p C_p + F_i C_i = \frac{B}{f} \qquad (5)$$

where a_p, a_i, and A have been previously defined; C_i is the concentration of interfering acid in the final solution measured; F_p and F_i are the factors which convert phthalic acid and interfering acid, respectively, to their potassium salt equivalents as they occur in the Kappelmeier precipitate; B is the weight of the Kappelmeier precipitate; and f is the aliquot factor involved in the procedure.

The above pair of equations assumes complete solubility of the interfering acid on acidification of the dissolved potassium salts.

Table II. Analysis of Synthetic Acid Mixtures (*1*)

	Composition of Mixture		Phthalic Found, Wt. %			Error,
	Phthalic, wt. %	Succinic, wt. %	1	2	Av.	%
1	5.74	94.26	5.80	5.90	5.85	+0.11
2	25.82	74.18	25.83	25.75	25.79	−0.03
3	51.47	48.53	51.55	51.63	51.59	+0.12
4	75.91	24.09	76.10	76.04	76.07	+0.16
5	95.60	4.40	95.38	95.46	95.42	+0.18
		Adipic				
1	5.12	94.88	5.22	5.36	5.29	+0.17
2	27.49	72.51	27.29	27.33	27.31	−0.18
3	50.21	49.79	50.29	50.19	50.24	+0.03
4	75.46	24.54	75.17	75.27	75.22	−0.24
5	94.72	5.28	94.44	94.60	94.52	−0.20
		Sebacic				
1	6.43	93.57	6.65	6.53	6.59	+0.16
2	26.14	73.86	26.40	26.22	26.31	+0.17
3	50.56	49.44	50.27	50.35	50.31	−0.25
4	74.92	25.08	74.65	74.69	74.67	−0.25
5	96.47	3.53	96.28	96.12	96.20	−0.27
		Itaconic				
1	5.79	94.21	5.62	5.67	5.65	−0.14
2	25.33	74.67	25.44	25.53	25.49	+0.16
3	52.66	47.34	52.66	52.77	52.72	+0.06
4	75.10	24.90	75.00	74.90	74.95	−0.15
5	95.28	4.72	95.07	95.03	95.05	−0.23
		Maleic				
1	97.79	2.21	97.78	97.90	97.84	+0.05
2	92.73	7.27	92.62	92.46	92.54	−0.19
3	84.86	15.14	84.81	84.69	84.75	−0.11
		Fumaric				
1	98.02	1.98	98.40	98.24	98.32	+0.30
2	93.71	6.29	93.56	93.74	93.65	−0.06
3	85.17	14.83	84.79	84.79	84.79	−0.38

Table III. Analysis of Phthalate Esters

	Sample	Phthalic Anhydride, % (Kappelmeier Method)			Phthalic Anhydride, % (Spectrophotometric Method)			Error,
		1	2	Av.	1	2	Av.	%
1	Oil-modified alkyd resin	35.85	35.95	35.90	35.98	36.06	36.04	+0.14
2	Dimethyl phthalate	75.48	75.62	75.55	75.60	75.60	75.60	+0.05
3	Dibutyl phthalate	53.24	53.18	53.21	53.17	53.21	53.19	−0.02
4	Dioctyl phthalate	38.40	38.42	38.41	38.57	38.81	38.69	+0.28

RESULTS AND DISCUSSION

Before the method was applied to esters, it was evaluated on synthetic mixtures of phthalic acid with various other polycarboxylic acids. All analyses were made in the presence of approximately 0.1 N hydrochloric acid, the technique being identical with that described above for the aqueous extracts of the Kappelmeier precipitates. In the case of

Table IV. Analysis of Synthetic Ester Mixtures (*1*)

Sample		P.A. Present, %[a]	P.A. Found, % (Spectrophotometric) 1	2	Av.	Error, %
Oil-modified alkyd + adipate polyester	1	34.20	34.01	34.19	34.10	−0.10
	2	25.14	25.12	24.82	24.97	−0.17
	3	18.61	18.55	18.29	18.42	−0.19
	4	8.57	8.10	8.31	8.21	+0.36
	5	2.07	2.40	2.18	2.29	+0.22
Oil-modified alkyd + sebacate polyester	1	33.71	33.71	33.51	33.61	−0.10
	2	23.82	23.51	23.68	23.60	−0.22
	3	17.94	18.29	17.95	18.12	+0.18
	4	7.76	7.99	7.85	7.92	+0.16
	5	3.16	3.27	3.57	3.42	+0.26
Dimethyl phthalate + dimethyl succinate	1	68.21	68.72	68.51	68.62	+0.41
	2	52.42	52.42	52.20	52.31	−0.11
	3	39.86	39.76	39.86	39.81	−0.05
	4	21.32	21.30	21.04	21.17	−0.15
	5	5.27	5.51	5.31	5.41	+0.14
Dimethyl phthalate + dimethyl sebacate	1	68.26	67.91	68.11	68.01	−0.25
	2	51.51	51.66	51.42	51.54	+0.03
	3	35.92	35.86	35.72	35.79	−0.13
	4	21.98	21.60	21.94	21.77	−0.21
	5	6.04	5.76	5.99	5.88	−0.16
Dimethyl phthalate + dibutyl maleate	1	71.74	71.79	71.60	71.69	−0.05
	2	65.41	65.33	65.07	65.20	−0.21
	3	60.82	60.94	61.24	61.09	+0.27
Dimethyl phthalate + dimethyl adipate	1	67.15	67.15	67.33	67.24	+0.09
	2	54.12	54.40	54.24	54.32	+0.22
	3	36.54	36.42	36.24	36.33	−0.21
	4	20.82	20.91	21.08	21.00	+0.18
	5	4.93	5.19	5.32	5.26	+0.33

[a] Calculated from phthalic anhydride content of phthalate ester present as determined by Kappelmeier method.

mixtures containing slightly soluble acids such as sebacic, the solutions were clarified when necessary, before making the absorbance measurements. Equations 4 and 5 were used in calculating results on the phthalic-fumaric and phthalic-maleic mixtures. Results on the acid mixtures studied are given in Table II. Table III shows the results obtained on an oil-modified alkyd resin and three monomeric phthalate esters. Table IV gives results for mixtures of phthalate esters with those of other polycarboxylic acids. Tables V and VI illustrate the applicability of the method to samples containing styrene and vinyl resins.

Table V. Analysis of a Styrenated Alkyd and Alkyd-Polystyrene Blends

Sample	P.A. Present, %	P.A. Found, % (Spectrophotometric) 1	2	Av.	Error
Oil-modified alkyd + polystyrene					
1	26.70[a]	26.39	26.69	26.54	−0.16
2	29.80[a]	29.91	29.71	29.51	−0.29
Styrenated alkyd					
1	29.37[b]	27.64	27.95	27.79	

[a] Calculated from phthalic anhydride content of alkyd as determined by Kappelmeier method.
[b] Synthetic value.

Table VI. Analysis of Vinyl Resin–Phthalate Ester Blends

Sample	P.A. Present, %	P.A. Found, % (Spectrophotometric) 1	2	Av.	Error
Polyvinyl chloride acetate + dibutyl phthalate	13.44[a]	13.85	13.57	13.71	+0.27
Polyvinyl chloride acetate + dioctyl phthalate	9.54[a]	9.15	9.33	9.24	−0.30

[a] Calculated from phthalic anhydride content of phthalate ester present as determined by Kappelmeier method.

It is evident from the data that accuracy and precision are good in all cases. While the chief advantage of the method is realized when it is applied to mixtures that cannot be analyzed by the Kappelmeier procedure, it also offers some advantages over the latter procedure in the case of a straight alkyd resin or other straight phthalate ester. The somewhat controversial question as to the best weighing form of the precipitated phthalate salt is obviated and the probability of error due to small amounts of unsuspected materials which may appear in this precipitate is minimized. Furthermore, the method is free from the so-called "carbonate error," characteristic of the Kappelmeier procedure, because potassium carbonate does not interfere. It will probably be possible to eliminate or greatly shorten the drying time required for the Kappelmeier precipitate, although the drying step is included at present before extracting with water.

The value of a_i (absorptivity of maleic acid) used in calculating results on the phthalate-maleate mixtures (Table IV) was obtained by saponifying dibutyl maleate under the conditions of the analytical procedure, dissolving and acidifying a weighed sample of the precipitated salt, and measuring the absorbance after suitable dilution. Measurement of a_i in this manner, rather than on a solution of pure maleic acid, minimizes the possibility of error which may rise from isomerization of some maleic to fumaric in the saponification process. Such error should be small, as the absorptivity values for maleic and fumaric acids at 276 mμ are low and do not differ greatly.

Results on the phthalic-maleic acid mixtures (Table II) indicate no apparent decrease in accuracy up to 15% maleic. Mixtures containing larger proportions of maleic can be analyzed, but accuracy will decrease with increasing maleic content. In general, maleic acid as such is seldom used in large amounts in finishes; furthermore, when present it is often "adducted" to drying oils, rosin, etc., in which case little if any potassium maleate will appear in the Kappelmeier precipitate.

As indicated in Tables V and VI, the method is not limited to ester mixtures. The Kappelmeier precipitate from a styrenated alkyd resin or an alkyd-polystyrene blend always contains polystyrene; that from a phthalate plasticized vinyl resin contains a mass of vinyl degradation products and potassium chloride. In the case of the styrene types the phthalic can be estimated by a modification of the Kappelmeier method; such an estimate in the presence of vinyl resins, however, is very difficult to obtain by conventional methods. In either case reliable results are rapidly obtained by the new method.

The various acids thus far studied include most of the types likely to be encountered in analysis of finishes. In addition to those for which analytical data are given, the authors have measured the absorption of diglycolic, oxalic, tartaric, and citric acids. These measurements indicate that none of these will cause serious interference. Although only three acids in the saturated dibasic series have been studied (those more commonly used in finishes), it is safe to predict that saturated dibasic acids as a class will not interfere. Most aromatic polycarboxylic acids will interfere seriously, because they appear in the Kappelmeier precipitate and absorb strongly in the ultraviolet. Although few aromatic polycarboxylic acids other than *o*-phthalic are as yet widely used in finishes, such acids as terephthalic, isophthalic, and methylene disalicylic may occasionally be encountered. Terephthalic acid can be largely removed owing to its very low water solubility, but the low concentration remaining will still cause interference. Other types of interfering materials will occasionally be encountered and separation of these may be required.

In the case of complete unknowns, the original sample and/or the aqueous extract from the Kappelmeier precipitate must, of course, be examined qualitatively before the method can be applied with confidence. In such cases the complete ultraviolet spectrum of the extract, together with various chemical tests for interfering materials likely to be present, will indicate whether the method is directly applicable or whether suitable modifications should be sought. The authors have found infrared absorption spectroscopic examination of the original sample and the precipitated salts from saponification (as a Nujol mull) to be very useful in this connection. Stafford *et al.* (*5*) have suggested the use of the infrared spectra of the dibenzyl amides of the dibasic acids as a means of determining what dibasic acids are present.

Although the method was primarily developed for use in the analysis of surface coatings, it should have useful applications in other fields.

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Residual Monomer in Polystyrene

Spectrophotometric Analysis

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A rapid method for the determination of monomeric styrene in polystyrene is described. Styrene absorbs ultraviolet radiation of wave lengths from 250 to 260 mμ, 40 to 100 times as intensely as polystyrene. Determination of small amounts of monomer in polymer, 0.1 to 2.0%, with precision and accuracy suitable for routine analysis, has been accomplished by absorption spectrophotometry. The method is most accurate in the absence of impurities other than styrene monomer. The presence of significant amounts of interfering substances is made evident by poor agreement of calculations for monomer from absorption measurements at three wave lengths.

THE pronounced effect of residual monomer on the physical properties and molding characteristics of commercial polystyrene makes the determination of residual monomer in the polymer important.

On account of the tenacious retention of styrene by polystyrene, especially by large particles, the usual determination of total volatile matter by treatment with heat and vacuum often gives low results. In a single determination, the frozen benzene technique of Lewis and Mayo (*4*) removed the styrene quantitatively but left in its place a few tenths of a per cent of benzene. Because of the difficulty in removing the styrene from its polymer in a state suitable for titration or precipitation, the methods of Kolthoff and Bovey (*3*), or Bond (*1*), are not easily applied to the determination of monomer in polymer.

Styrene absorbs ultraviolet radiation of wave lengths of 282 and 291 mμ, whereas polystyrene has only general absorption in this region of the spectrum. Owens (*7*) made use of this difference to analyze monomer in partially polymerized styrene, using a medium quartz spectrograph. McGovern, Grim, and Teach (*5*) adapted this method to a spectrophotometer and investigated variables affecting the analysis.

At a wave length of 251 mμ, styrene absorbs ultraviolet radiation 15 times as intensely as it does at 282 mμ, and 25 times as intensely as at 291 mμ. At this wave length it also has 100 times the absorption of polystyrene. Therefore conditions are favorable for the determination of small amounts of styrene in polystyrene by absorption measurements in this spectral region. Owing to the relative intensities with which styrene absorbs at 251, 282, and 291 mμ, interference by other materials is likely to be much less at 251 mμ than at the other wave lengths. In addition, the low concentration of polymer solution necessary for absorption measurements at 251 mμ is likely to cause less error from light scattering than the more highly concentrated solutions required for absorption measurements at longer wave lengths. This paper presents a spectrophotometric method of analysis of styrene in polystyrene by absorption measurements in the 251 mμ region. As in the methods of Owens (*7*), and McGovern *et al.* (*5*), multiple wave length determinations show the presence of interfering substances.

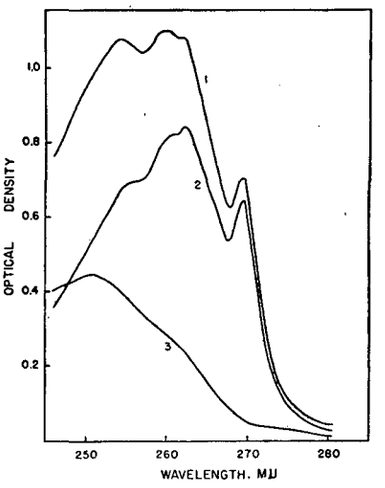

Figure 1. Ultraviolet Absorption Spectrum of Commercial Polystyrene

Curve 1. Before purification
Curve 2. After precipitation in alcohol
Curve 3. Difference between curves 1 and 2

The absorption near 250 mμ of a mixture of styrene and polystyrene can be resolved by simple mathematics into the absorption of the styrene and that of polystyrene. However, to give significant results, two conditions must be met. The optical interference of impurities other than styrene must be low enough

Table I. Preparation of Polystyrene Samples

Sample	Monomer Preparation	Polymerization Conditions	Catalyst	Molecular Weight[a]
A	Crystallized	Heated in vacuo	None	950,000
B	Distilled	Standing at room temperature	None	590,000
C	Fractionally distilled	Ultraviolet irradiated	0.3% biacetyl	540,000
D	Commercial, not purified	Emulsion, heat	0.3% sodium persulfate	370,000
E	Commercial	Commercial polymer	Not known	170,000

[a] From intrinsic viscosity measurements (*2*).

that no significant error results. Secondly, the absorption spectrum of polystyrene must be constant—that is, independent of molecular weight, conditions of polymerization, catalyst, or other variables.

In Figure 1 the absorption spectrum of a solution of commercial polystyrene is shown to be a composite of the spectra of pure polystyrene and styrene. Curve 1 is the absorption spectrum of a commercial polystyrene solution in chloroform. Curve 2 is the spectrum of an equal concentration of the same polymer, purified by precipitation from chloroform solution with alcohol. Curve 3 is the difference between curves 1 and 2. Within experimental error, curve 3 is the absorption spectrum of the impurities removed in the purification process, and is identical to the absorption spectrum for styrene which is shown in Figure 2. Although there are, no doubt, small quantities of dimer, trimer, ethylbenzene, and other impurities in the polymer, their concentration and absorption are so low that the major ultraviolet-absorbing impurity is monomer. This is in accord with the reports of other workers (*5*, *6*). Identification of the styrene as the major impurity has been accomplished for several samples of commercial polystyrene from each of three major manufacturers, even for samples with as little as 0.1% residual monomer.

If the complete absorption spectrum of the sample solution is not measured, and the calculation of per cent monomer is made from absorbance measurements taken at a single wave length, all radiation-absorbing impurities are expressed as styrene—an assumption that may not always be justified. To retain rapidity of analysis, and also make known the presence or absence of other impurities, it is advisable to take absorbance measurements at several wave lengths. The absence of interference is indicated by the apparent per cent monomer being independent of the wave length at which the absorption measurement is made. When an interfering substance is present, the difference between its absorption spectrum and that of styrene is critical in causing poor relative agreements in the values for monomer content derived from multiple absorption readings. Additional factors affecting the disagreement and accuracy are the concentration and intensity of absorption of the foreign material.

The wave length region most suitable for absorption measurements is from 250 to 260 mμ, inasmuch as the chloroform solvent absorbs increasingly at shorter wave lengths and styrene becomes transparent rapidly at longer wave lengths. Fortunately, the location of the absorption maxima for styrene and polystyrene makes this region particularly favorable. In the method described, three wave lengths were used, 250, 255, and 260 mμ.

THEORY

If a sample of polystyrene weighing W grams containing X% residual monomer is dissolved in 1 liter of optically transparent solvent, the optical density, OD, of the solution will be

$$OD = \left(\frac{100-X}{100}\right) WE_p + \frac{X}{100} WE_m \quad (1)$$

from which

$$X = 100\left(\frac{E - E_p}{E_m - E_p}\right) \quad (2)$$

where E, E_p and E_m are the specific extinction coefficients of the sample, monomer-free polymer, and monomer, respectively.

EXPERIMENTAL

In the investigation to find whether the absorption spectrum of polystyrene was dependent upon any variables of the polymerization reaction, and in the measurement of the coefficient, E_p, five samples of polystyrene were used. The methods of preparation of these polymers are summarized in Table I. Each of these polymers was made monomer-free by repeated solution in chloroform and precipitation as a very fine filament by extrusion through a 0.4-mm. capillary into alcohol. In order to avoid loss of the low-molecular-weight fraction of the polymer, the volume of alcohol was always at least ten times that of the chloroform solution. Each polymer was finally vacuum dried for 16 hours.

Accurately weighed samples of approximately 0.12 gram of each polymer were dissolved in C.P. chloroform and the solutions were diluted to 250 ml. in volumetric flasks. The optical densities of these solutions at 250, 255, and 260 mμ were measured. A Beckman spectrophotometer was used, with 1.00-cm. silica cells and a slit width of 0.9 mm. at 250 mμ and 0.8 mm. at 255 and 260 mμ. The specific extinction coefficients were calculated in the usual way; concentrations were in grams per liter. By calibration with a mercury vapor lamp, the wave-length scale of the spectrophotometer was known to be accurate to ± 0.1 mμ.

The ultraviolet absorption spectra of the six polystyrenes were almost identical. There were reproducible small differences which were probably due to variations in degree of light scattering by the polymer solutions, or perhaps absorption of ultraviolet radiation by terminal groups in the polymer molecules. However, for purposes of this analysis, the spectrum of polystyrene may be considered constant. A summary of ten deter-

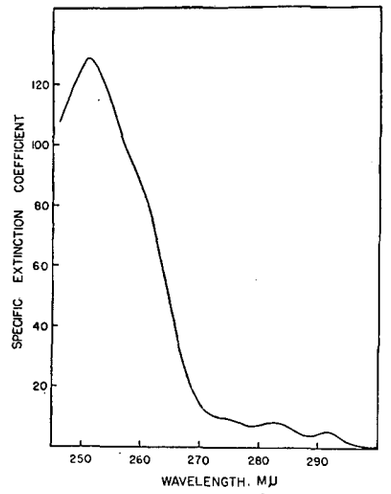

Figure 2. Ultraviolet Absorption Spectrum of Styrene Monomer

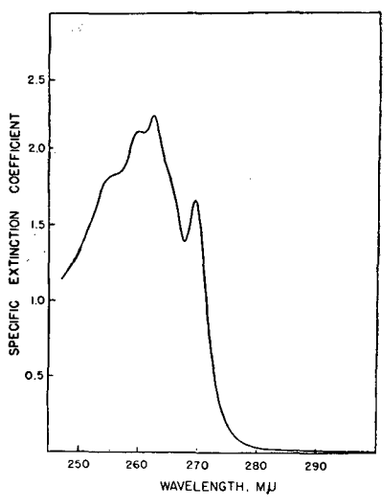

Figure 3. Ultraviolet Absorption Spectrum of Monomer-Free Polystyrene

Table II. Summary of Extinction Coefficients for Six Polystyrenes

	Wave Length, Mμ		
	250	255	260
Highest value	1.38	1.84	2.16
Lowest value	1.29	1.77	2.08
Average value	1.34	1.80	2.12
Standard deviation	0.030	0.024	0.024

Table III. Specific Extinction Coefficients of Polystyrene and Styrene

Wave Length, Mμ	Polystyrene, E_p	Styrene, E_m
250.0	1.34	136
255.0	1.80	116
260.0	2.12	87

Table IV. Per Cent Monomer in Synthetic Mixture of Purified Polystyrene and Styrene

Actual Styrene, %	Wave Length, Mμ 250.0	255.0	260.0	Average	Error
0.00	0.02	0.02	0.02	0.02	+0.02
0.14	0.15	0.16	0.17	0.16	+0.02
0.28	0.29	0.30	0.29	0.29	+0.01
0.56	0.56	0.57	0.56	0.56	0.00
				Av.	+0.01

Table V. Per Cent Monomer in Various Polystyrenes

Sample	Wave Length, Mμ 250.0	255.0	260.0	Average
Experimental	0.31	0.34	0.31	0.32
A-1	0.75	0.80	0.77	0.77
A-2	1.04	1.00	0.94	0.99
	1.18	1.13	1.07	1.13
B-1	0.80	0.80	0.79	0.80
	0.79	0.81	0.77	0.79
B-2	0.42	0.42	0.40	0.41
	0.45	0.44	0.42	0.44
B-3	0.16	0.12	0.09	0.13
	0.19	0.15	0.11	0.15
B-4	0.09	0.10	0.10	0.10
C-1	0.20	0.18	0.15	0.18
	0.22	0.19	0.17	0.19
C-2	1.05	1.04	1.00	1.03
	1.58	1.57	1.54	1.56

minations of the extinction coefficients at 250, 255, and 260 mμ is given in Table II.

The standard deviations of the extinction coefficients, when expressed as equivalent monomer content, are 0.02% at 250 and 255 mμ, and 0.03% at 260 mμ. The absorption spectrum of monomer-free polystyrene is shown in Figure 3.

The sample of styrene used in the determination of the specific extinction coefficient, E_m, was distilled at 50° C. under 20-mm. vacuum. Only the mid-fraction was used. The absorption spectrum of styrene in chloroform solution is given in Figure 2. The specific extinction coefficients of polystyrene and styrene from 250 to 260 mμ are given in Table III.

When the numerical values for E_p and E_m are substituted in Equation 2, the following equations result:

$$\text{At } 250 \text{ m}\mu,\ \%\ \text{styrene} = 0.743\ (E_{250} - 1.34) \quad (3)$$

$$\text{At } 255 \text{ m}\mu,\ \%\ \text{styrene} = 0.876\ (E_{255} - 1.80) \quad (4)$$

$$\text{At } 260 \text{ m}\mu,\ \%\ \text{styrene} = 1.178\ (E_{260} - 2.12) \quad (5)$$

The purification process for polystyrene removes quantitatively the monomer and other alcohol-soluble impurities. The spectrophotometric analysis of mixtures of purified polymer and known amounts of monomer does not prove the accuracy of this method in the presence of interfering impurities. However, it does show the precision to be expected, and the accuracy in the absence of interferences.

A weighed sample of a purified polymer was dissolved in chloroform, and aliquots were mixed with varying amounts of a 0.00751-gram-per-liter styrene solution. The per cent styrene was calculated on total solute. The results of spectrophotometric analysis of the solutions and calculation of results using Equations 3 to 5 are shown in Table IV.

Several experimental polymers and eight commercial polymers have been analyzed with the spectrophotometer. The results of some of these analyses are shown in Table V. Sample weights were approximately 0.12 gram, and solution volume was 250 ml. The commercial samples were from three manufacturers (letters A, B, and C in the sample designation), and were in the form of beads, clear cubes, or opaque chips.

DISCUSSION OF RESULTS

The results in Table IV indicate that in the absence of interfering impurities the absolute accuracy of the method is approximately ± 0.03%, and the absolute precision is about ± 0.02%. The analytical results for some commercial samples are less accurate and precise.

In Table V, the results for the duplicate determinations of monomer in samples A-2 and C-2 are in poor agreement. In these two cases the polymer sample was in the form of coarse chips or cubes, and the variation in monomer in the particles was great enough that the 0.12-gram sample did not represent the whole material under examination. No doubt better agreement of duplicate determinations could have been achieved by dissolving much larger amounts of polymer, and diluting aliquots to suitable concentrations. On the other hand, by using smaller sample weights (minimum 1 mg.) and correspondingly small solution volumes (minimum 3 ml.), it is possible to determine the styrene content in individual polymer chips when the distribution of monomer among the different particles is of interest.

The results for monomer content for the experimental polymer, and for A-1, B-1, B-2, and B-4 agree so well at the three wave lengths that it is likely that the values in the average column are accurate. On the other hand, the results for A-2, B-3, C-1, and C-2 indicate the presence of interfering material. In these cases, the percentages of monomer are high. These results may still be significant when it is considered that the lowest per cent shown for each represents its maximum possible monomer content—for example, samples B-3 and C-1 have monomer contents of 0.11% or less, and 0.17% or less, respectively. This information may be sufficient for some purposes in quality control.

SUMMARY

The spectrophotometric method of analysis of polystyrene for residual monomer has several advantages, and some limitations. No treatment of sample other than solution is required. The method is rapid, and has precision and accuracy suitable for quality control. However, the method is limited to polymers which have no pigments or dyes. Agreement of results at multiple wave lengths shows when interfering materials are present. In the presence of interference, only maximum monomer content can be determined.

ACKNOWLEDGMENT

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Spectrophotometric Determination of Nickel in Calcium Metal

Using 1,2-Cyclohexanedionedioxime

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In the course of searching for a method that would be satisfactory for the routine determination of nickel in calcium metal containing more than trace amounts of iron, the possibility of using the *vic*-dioximes was investigated. The reactions of 1,2-cyclohexanedionedioxime (nioxime) and 1,2-cycloheptanedionedioxime (heptoxime) with nickel in the presence of an oxidizing agent in alkaline solution are similar to the reaction of dimethylglyoxime in that, in each case, unstable, reddish-brown colors are produced having absorption spectra which are very similar. However, the red inner complex compound of nickel(II) with 1,2-cyclohexanedionedioxime can be stabilized with gum arabic, is insensitive to pH changes, absorbs most strongly at 550 mμ, and conforms to Beer's law. The applicability of this method to the spectrophotometric determination of nickel has been established. The effects of diverse ions are discussed and the method is applied to the determination of nickel in calcium metal.

APPROXIMATELY 125 papers published since 1900 have dealt with the colorimetric, photometric, or spectrophotometric determination of nickel. Hydrogen sulfide (*10*), potassium thiocarbonate (*13*), and ammonia (*4*) are inorganic reagents which have been proposed. Organic reagents have proved to be more sensitive, and the majority of the colorimetric methods suggested for the determination of nickel were based on its reactions with such compounds. Various authors have investigated the sodium salt of diethyl-dithiocarbamate (*2*), potassium dithiooxalate (*7*), 8-hydroxyquinoline (*18*), triethanolamine (*11*), formaldoxime (*8*), and 3-nitrososalicyclic acid (*20*).

The most widely used reagents for nickel have been the *vic*-dioximes—i.e., compounds containing the group —C(=NOH)—C(=NOH)—, which reacts selectively with nickel and other transition group metals. The gravimetric procedures involving these reagents are well known (*6*), and recently Voter and Banks have discussed the use of some water-soluble dioximes (*24*).

Colorimetric procedures employing the *vic*-dioximes are also widely used. The American Society for Testing Materials procedure employs α-furildioxime; the nickel complex is formed in aqueous solution and is extracted into *o*-dichlorobenzene for the colorimetric comparison or measurement (*3*).

The reaction of nickel with dimethylglyoxime in the presence of powerful oxidizing agents in strongly basic solutions has been studied and used widely since its value as a means of quantitatively determining nickel was pointed out by Rollet (*22*). While the intensely colored (reddish-brown) complex is potentially an excellent colorimetric form, its instability and the drastic conditions for its formation are disadvantageous. Although over sixty published papers have discussed Rollet's method and recent spectrophotometric investigations have suggested improvements (*9*, *17*), the exact nature of the reaction is still unknown. Consequently, the method is largely empirical, and a closely standardized procedure must be used (*9*). A similar reaction with α-furildioxime has been reported, but the complex was too unstable for quantitative work (*16*). Johnson and Simmons (*12*) recently reported the same type of reaction for nioxime (1,2-cyclohexanedionedioxime). They preferred to stabilize the nickelous nioxime complex with gum arabic, however; and they used the latter form for a photometric method of determination.

Because the *vic*-dioximes offered a number of good possibilities, an examination of the colorimetric properties of their nickel complexes was undertaken.

APPARATUS

Constant wave-length measurements of the solutions were made with a Beckman quartz photoelectric spectrophotometer, Model DU. The wave-length studies were made with a Cary automatic recording photoelectric spectrophotometer, Model 12. Matched sets of 5.000-cm. and 1.000-cm. Corex cells were used for the solutions and blanks. Corrections for slight differences in the transmittancies of the cells were made whenever necessary.

The pH measurements were made with Beckman Model H-2 and Model M pH meters.

REAGENTS

Bromine water, saturated.

Calcium chloride solution. A solution of the reagent-grade salt was treated with nioxime and activated charcoal to remove traces of iron and nickel.

Diammonium citrate solution, 20%.

Dimethylglyoxime solution, 1% in 95% ethyl alcohol.

Gum arabic solution, 10%.

Heptoxime (1,2-cycloheptanedionedioxime, Hach Chemical Co., Ames, Iowa) solution, 0.47%.

Nickel solution. A stock solution was prepared from Mond nickel dissolved in aqua regia. Appropriate dilutions were used as the standard nickel solutions for the preparation of calibration curves.

Nioxime (1,2-cyclohexanedionedioxime) solution, 0.8%.

Reagent-grade chemicals were used throughout the work. Further purification was carried out whenever necessary. The acids and bases used were the C. P. grade ordinarily used in the analytical laboratory.

COLOR REACTIONS

Preliminary experiments with the reaction of nickel with dimethylglyoxime, using bromine water as the oxidizing agent, confirmed the reported instability of the oxidized complex. Typical absorption spectra obtained for the complexes which develop in ammoniacal solutions (pH 11.3) are given in Figure 1. Curves 1 and 2 were obtained 10 and 60 minutes after mixing, respectively. Sets of curves obtained at 10-minute intervals were nearly identical to those previously reported in the literature (*14*, *17*, *19*).

Attempts to improve the stability of the oxidized complex were not uniformly successful. The addition of sodium hydroxide, as recommended by Makepeace and Craft (*14*), increased the rate of fading. Makepeace and Craft attributed this behavior to impurities in the reagents, and recommended special precautions for the preparation of the solutions to be used. More recent papers, notably that of Furman and McDuffie (*9*), have suggested further modifications which reportedly produce more stable products. Investigation of these reactions is to be treated in later work.

Because heptoxime and nioxime (*24*) have certain advantages over dimethylglyoxime, it was of interest to study their reactions with nickel in the presence of oxidizing agents. Both heptoxime and nioxime produced reddish-brown complexes nearly identical

to the dimethylglyoxime complexes. When they reacted with nickel in the presence of bromine water and ammonium hydroxide, the three *vic*-dioximes produced absorption spectra which were very nearly superimposable throughout the visible range. Because the heptoxime and nioxime complexes were also unstable under these conditions, the new reagents appeared to produce no improvement in Rollet's method.

3 and 4 of Figure 2. The suspensions contained 1 and 4 ml. of gum arabic solution, respectively, and were at pH 9. The absorbancies of these solutions continued to increase until they reached the value obtained for solution 1.

The results in basic solutions were more variable, undoubtedly because of the retardation of color development. The optimum pH range was from pH 4 to 6, although the complex could be

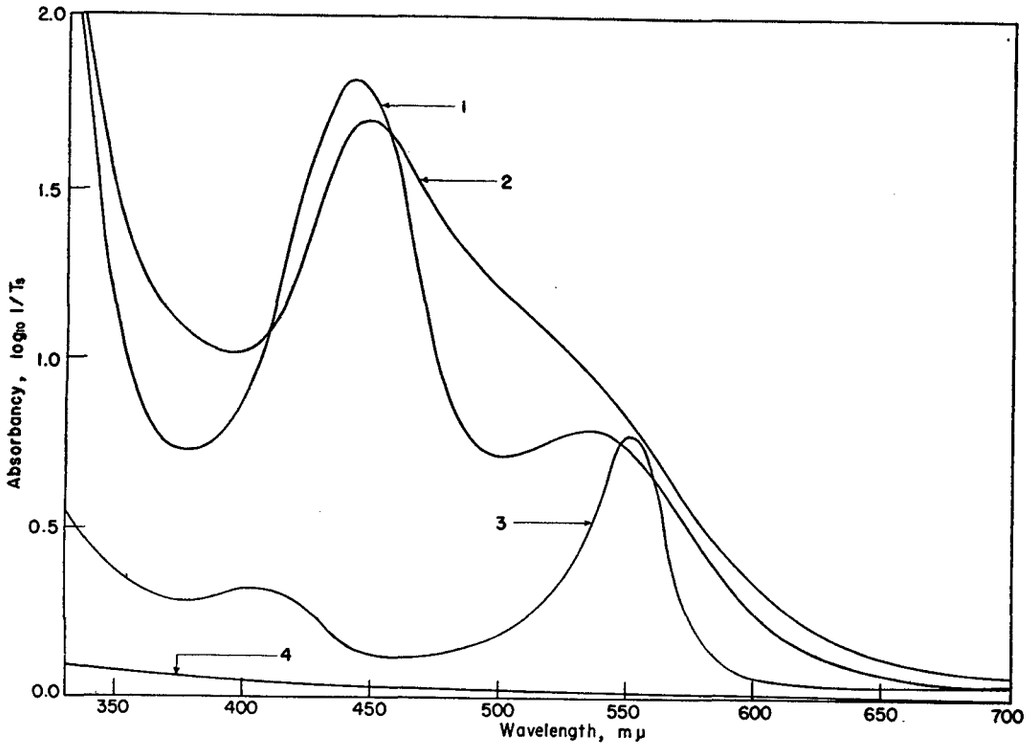

Figure 1. Absorption Spectra of Nickel Dioxime Solutions

154 γ of nickel per 100 ml.
Cary instrument, using 5.0-cm. cells
1, 2. Oxidized dimethylglyoxime complex at 10 and 60 minutes, respectively
3. Nickel nioxime, stabilized with gum arabic at 10 and 60 minutes
4. 1 ml. each of 0.8% nioxime and 10% gum arabic solutions diluted to 100 ml.

On the other hand, the formation of nickelous nioxime in the presence of gum arabic, according to the procedure of Johnson and Simmons (*12*), produced a highly colored, stable suspension. This red complex was the form selected for further spectrophotometric investigation.

COLORIMETRIC PROPERTIES OF NICKELOUS NIOXIME

Absorption Spectrum. A pronounced maximum in the absorption spectra of the suspensions (curve 3, Figure 1) occurred at 550 mμ. The wave length of the maximum was confirmed for a range of concentrations of nickel, using both the Cary and the Beckman spectrophotometers.

The reagents did not absorb appreciably in the visible region, as is shown by curve 4 of Figure 1. Excess nioxime had no effect on the color intensity, 10 ml. of the reagent producing the same intensity as 1 ml.

Stability. Although gum arabic stabilized the colored system, excess reagent retarded the rate of color development. Curves 1 and 2 of Figure 2 show the absorbancy at 550 mμ as a function of time for suspensions containing, respectively, 1 and 4 ml. of 10% gum arabic solution in a 100-ml. final volume. The solutions were at pH 5. The absorbancies of both suspensions became identical within 24 hours, and they remained constant for at least a week.

Effect of pH. The retardation of color development was much more pronounced in basic solutions, as indicated by curves

developed satisfactorily from pH 3 to 10. The color development in acidic solutions was essentially complete in 1 hour. Longer periods were necessary for suspensions prepared under basic conditions.

Calibration Data. The optimum range of concentrations was obtained directly from Figure 3, in which the percentage transmittancy was plotted as a function of the logarithm of concentration. This method is similar to that suggested by Ringbom (*21*), in which percentage absorptancy was plotted against the logarithm of concentration. In this type of plot, the range of maximum accuracy falls between 20 and 60% transmittancy. Thus, the optimum range was 2 to 7 micrograms of nickel per milliliter when 1.000-cm. cells were used, or 0.5 to 1.4 micrograms per milliliter in 5.000-cm. cells. The relative accuracy at a particular concentration can be determined from the slope of the curve (*21*).

Conformity to Beer's law was confirmed by applying the statistical treatment recommended by Mandel (*15*). The absorbancies of duplicate sets of suspensions, ranging from 0.1 to 1.4 micrograms of nickel per milliliter, were determined at 550 mμ. The least squares line, total variability, experimental error, and departure from linearity were calculated. The variance ratio, $F = 1.41$ with $n_1 = 12$ and $n_2 = 14$, proved to be much smaller than the value given by the F-table (*23*), in which $F(5\%) = 2.53$; therefore, there was no significant departure from linearity in the range studied.

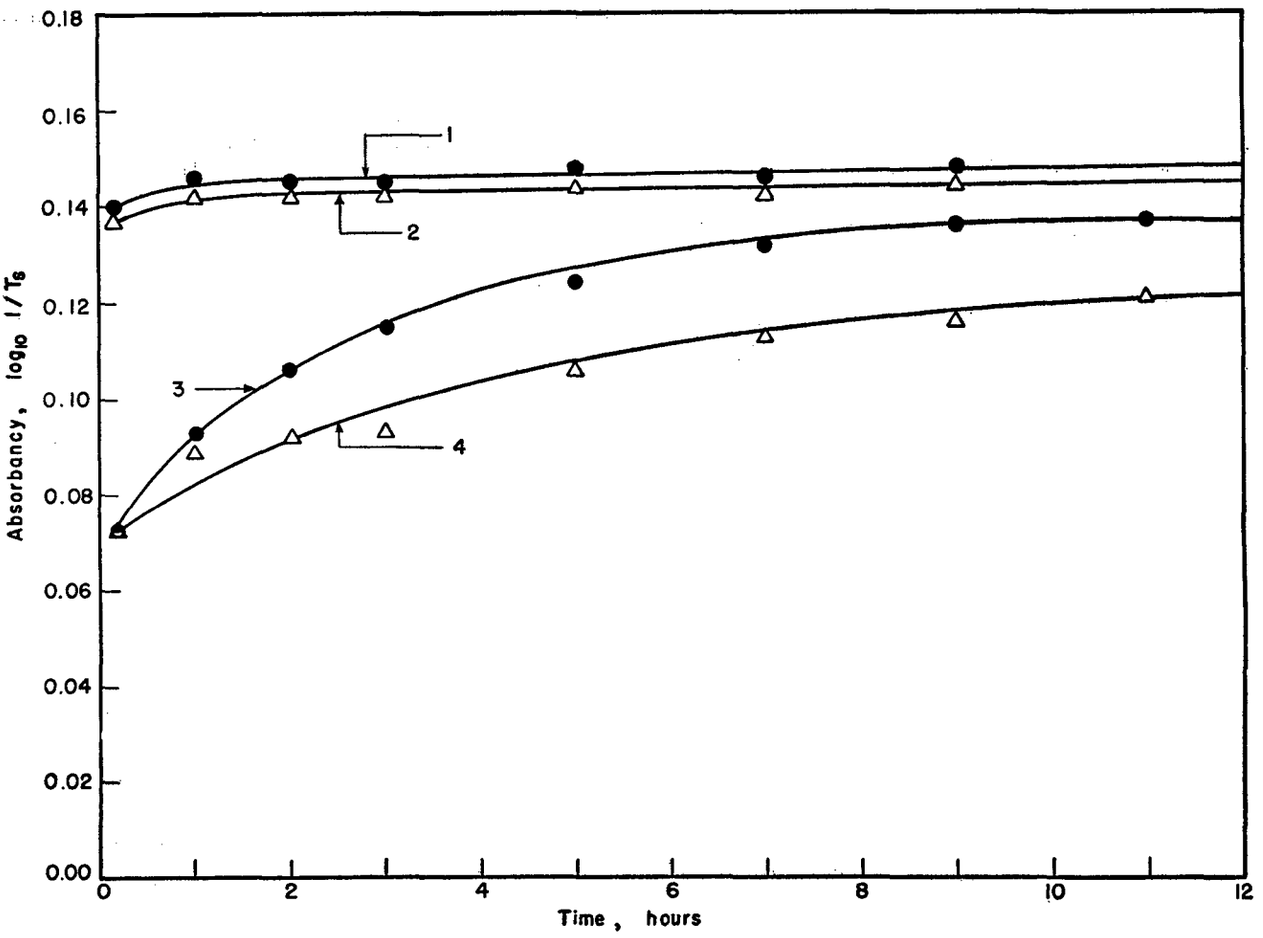

Figure 2. Stability of Nickelous Nioxime Suspensions

154 γ of nickel per 100 ml.
Beckman instrument, using 1.0-cm. cells.
Volume of 10% gum arabic solution:
1,3. 1 ml.
2,4. 4 ml.
1,2. pH 5
3,4. pH 9

The least squares line and the experimental points were plotted in Figure 4. The standard deviation of (*5*) absorbancy for a single analysis was 0.0049. "Confidence limits" at the 99% level were calculated from this standard deviation and Student's *t*-table (*23*).

Interferences. The Cary instrument was used to determine the effect of diverse ions on the absorbancies in the region of 550 mμ. Those ions which produced absorbancies outside of the confidence limits were treated as interferences.

Anions which did not interfere when present in concentrations 1000 times the nickel concentration were the acetate, arsenite, borate, bromate, bromide, carbonate, chlorate, chloride, citrate, dichromate, ferricyanide, fluoride, iodide, mandelate, molybdate, nitrate, nitrite, perchlorate, phosphate, silicate, sulfate, thiocyanate, thiosulfate, tungstate, and vanadate ions. Ferrocyanide, cyanide, and ethylenediamine tetraacetate ions prevented the formation of the color. Potassium iodate produced results which were 10% low. Hexanitratocerate formed a precipitate at the pH of the determination. The oxalate ion produced a cloudiness in the presence of gum arabic. The permanganate ion absorbed appreciably at 550 mμ and had to be absent.

The cations which did not interfere when the ratio of the ion to nickel was 1000 to 1 were the ammonium, sodium, potassium, beryllium, magnesium, calcium, strontium, barium, lanthanum, hydroxylammonium, manganous, zinc, cadmium, mercuric, aluminum, and plumbous ions. Several cations precipitated at the pH of the determination—e.g., titanium, zirconium, hafnium, vanadyl, ferric, stannous, and stannic. The colored chromic ion absorbed somewhat at 550 mμ and could be present in a ratio of no more than 50 to 1. The ferrous, cobaltous, and cupric ions reacted with nioxime to produce highly colored complexes which interfered.

Curve 3 of Figure 5 was obtained for a solution of the ferrous nioxime complex formed under acidic conditions. The interference was minimized by oxidizing the iron to the ferric state and complexing it with diammonium citrate. The absorption spectrum of the citrate complex has been given as curve 4 of Figure 5. Curves for nickelous nioxime (curve 1) and for Rollet's complex (curve 2) have been included for comparison. The slight interference due to ferric citrate can be further reduced by the use of a suitable blank.

The ferric citrate complex was slowly decomposed and the iron was reduced by excess nioxime. Therefore, a moderate excess of citrate is desirable. If the absorbancy measurements are to be made within an hour or two after mixing, 3 ml. of 20% diammonium citrate solution in 100 ml. of suspension are the upper limit permissible, as citrate has a retarding effect on the color development.

High concentrations of electrolytes caused the results to be consistently low. This manifestation of the diverse ion effect was eliminated by preparing the calibration suspensions at the ionic strength expected in the determinations.

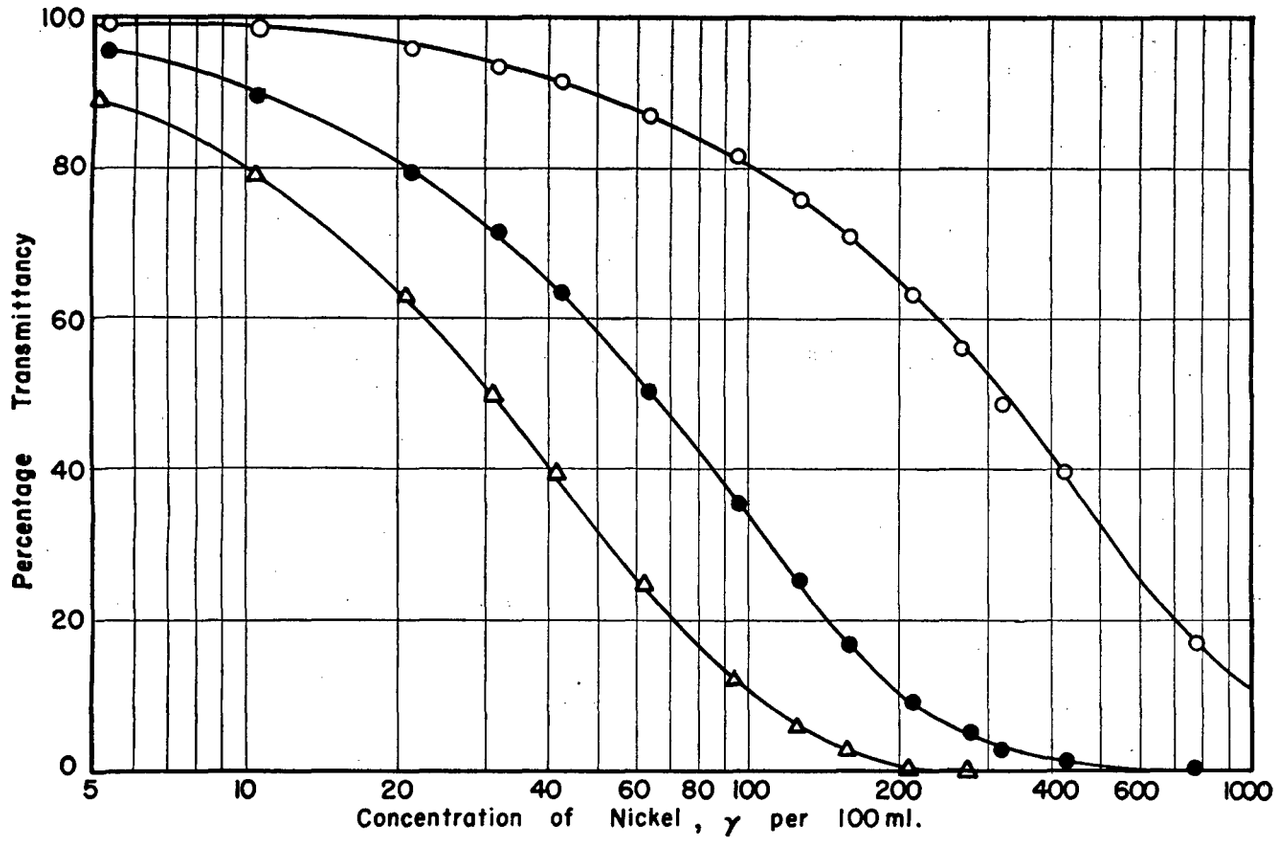

Figure 3. Calibration Curves for Nickelous Nioxime Suspensions

● 5-cm. cells, Beckman instrument △ 10.0-cm. cells, calculated
○ 1.0-cm. cells, calculated

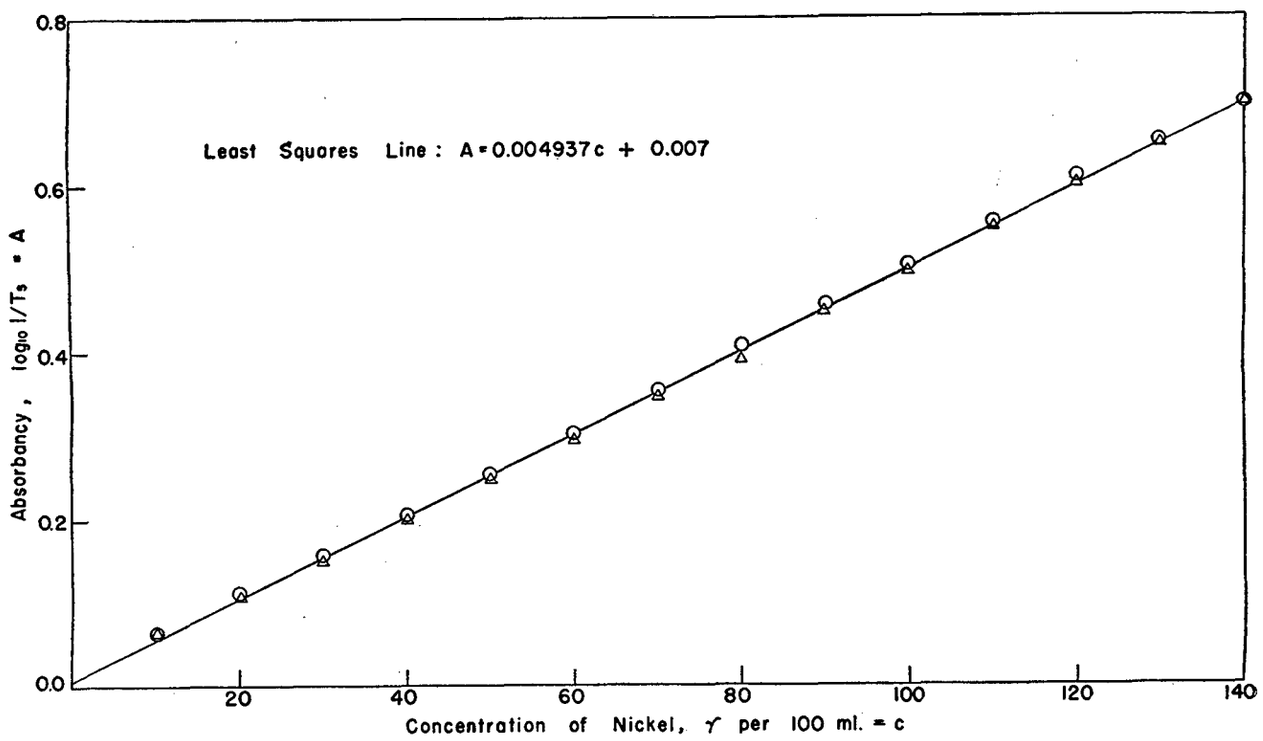

Figure 4. Least Squares Fit for Nickelous Nioxime Suspensions

Measured with Cary instrument, using 5.0-cm. cells. Duplicates run on separate days

Table I. Nickel in Calcium Solutions[a]

Nickel Taken, γ	Iron Added, Mg.	Nickel Found, γ
15	..	14
31	..	31
46	..	45
62	..	62
77	..	79
20	1.0	20
40	1.0	38
60	1.0	57
80	1.0	79
100	1.0	99

[a] Each solution contained 1.0 gram of calcium.

DETERMINATION OF NICKEL IN CALCIUM METAL

Abbey (*1*) has applied Rollet's method to the determination of traces of nickel in high purity calcium and magnesium metals. He was able to make the absorbancy measurements at 445 mμ because the iron concentration was so low that absorption by ferric citrate was negligible. Because the amount of iron in the metal is often many times that found in Abbey's samples, the absorbancies should generally be measured at 530 mμ. Reference to curves 1 and 2 of Figure 5 indicates that the absorbancy indexes at the lower peak of the oxidized complex and at the nickelous nioxime peak are nearly the same. Therefore, the loss in sensitivity in changing to the newer method is small.

Calibration Curve. Solutions containing 1 gram of calcium and known amounts of nickel were prepared by adding the standard nickel solution to appropriate volumes of the calcium chloride solution. These solutions, in 100-ml. volumetric flasks, were treated with 1 ml. of 20% diammonium citrate solution, 1 ml. of 10% gum arabic solution, and 1 ml. of 0.8% nioxime solution. The contents of the flasks were mixed after each addition and after dilution to volume. A blank solution was prepared in the same manner, omitting the nickel and the nioxime. In 1 to 2 hours the absorbancy measurements were made in 5.000-cm. cells, at 550 mμ and a 5 mμ nominal band width. The concentration range was 10 to 150 micrograms of nickel per 100 ml.

Calcium solutions containing known amounts of nickel and iron were analyzed to check the accuracy of the method. Table I gives the results obtained for a series of these solutions.

Recommended Procedure. Place 50 ml. of water in a 400-ml. beaker, and carefully add a weighed sample of 2 grams of small calcium particles. When the reaction is complete, add 5 ml. of concentrated hydrochloric acid and 1 ml. of concentrated nitric acid, and heat to dissolve the hydroxide and to oxidize any iron. Cool and add 2 ml. of 20% diammonium citrate, then adjust the solution to pH 4 to 6 with ammonium hydroxide. Rinse the solution into a 100-ml. volumetric flask, dilute to the mark, and mix. Pipet 50 ml. of this dilution into another 100-ml. flask, retaining the remainder for the blank. Add 1 ml. of 10% gum arabic solution to each flask, and mix well. Add 1 ml. of 0.8% nioxime solution to the first aliquot only, dilute both solutions to the mark, and mix well. Measure the absorbancy of the colored aliquot against the blank after an hour. The wave-length setting should be 550 mμ.

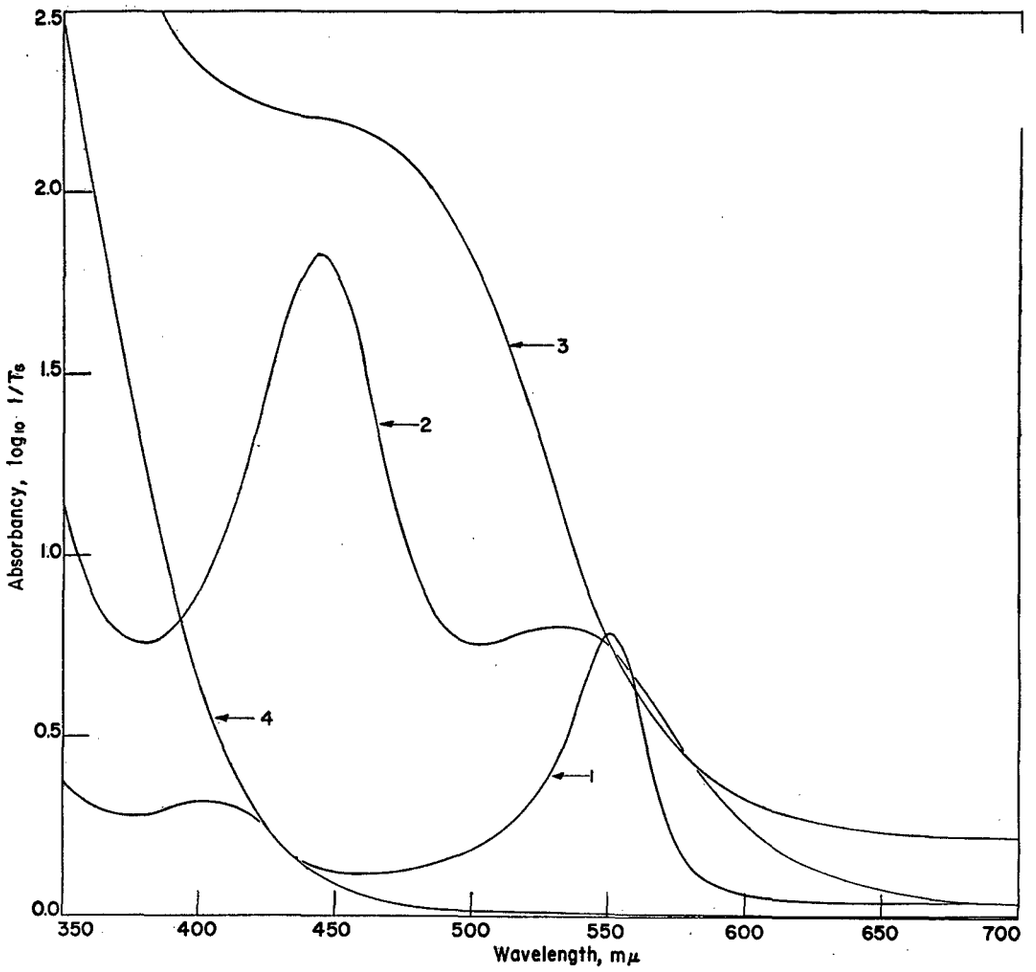

Figure 5. Absorption Spectra of Complexes of Iron

Volume of solution 100 ml.
Obtained with Cary instrument, using 5.0-cm. cells
1. Nickelous nioxime 154 γ of nickel present
2. Oxidized dimethylglyoxime complex at 10 minutes, 154 γ of nickel present
3. Ferrous nioxime, 500 γ of iron
4. Ferric citrate, 2000 γ of iron

The range of nickel concentration chosen will depend upon the cell thickness to be used. The order of addition of the reagents is not critical, provided that the nioxime is added last.

DISCUSSION

The nickelous nioxime method appears to have several advantages over the conventional dimethylglyoxime colorimetric procedure. The gum arabic suspensions are much more stable, although the maximum intensity is not reached immediately. The drastic conditions of Rollet's method cause most cations to precipitate, but the milder conditions of the new method permit the direct determination of nickel in the presence of several of these ions.

Although comparison of curves 1 and 2 of Figure 5 would indicate that Rollet's method is much more sensitive, the absorption of ferric citrate is usually so great that the smaller maximum at 530 mμ is used instead of the intense maximum at 443 mμ. The absorbancy index of nickelous nioxime at 550 mμ is of the same order as that of Rollet's complex at 530 mμ. On this basis, the sensitivities of the two methods are comparable.

The nickelous nioxime method has the great advantage of simplicity. It requires few preliminary separations and eliminates the extractions required in some procedures. The accuracy and reproducibility are sufficient for the purposes of trace analysis.

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Spectrophotometric Determination of Cerium(IV)

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It was desired to study the behavior of solutions of cerium at as low concentrations as could be conveniently determined. It appeared likely that the sensitivity of an existing colorimetric method, based on the yellow color of cerium(IV), could be improved by working in the ultraviolet. Cerium(IV) was found to show an absorption maximum at approximately 320 mμ, with a molar extinction coefficient of 5.58 × 10^3, in 1 *N* sulfuric acid. A photometric sensitivity of 0.025 microgram can thus be realized. In the determination of cerium by oxidation with persulfate, it is important not to take too large an amount of persulfate or of ammonium ion, because both residual persulfate, and nitrate ion formed by oxidation of ammonium ion, absorb appreciably at 320 mμ. A spectrophotometric procedure for the determination of traces of cerium improves sensitivity twenty-fold over the previous colorimetric procedure.

THE familiar yellow color of the ceric ion in acid medium has been made the basis for a colorimetric procedure, in which cerous ion is oxidized with persulfate in boiling 1 *N* sulfuric acid with silver ion as a catalyst (*8*). If the intensity of the yellow color is determined visually, the sensitivity is approximately 10 micrograms of cerium per square centimeter, while the photometric sensitivity with a blue filter is 0.5 microgram (*8*). A spectrophotometric study in the visible range (*2*) has established that the absorption of ceric sulfate increases continuously up to 480 mμ (the lower limit of the study), so that as pointed out by Sandell (*8*), increased sensitivity would be expected to result from use of monochromatic violet light. Since completion of the present work, Freedman and Hume (*1*) have shown that the maximum absorption occurs in the neighborhood of 315 mμ, and have reported on work in which cerium was oxidized by a modification of the procedure of Sandell, and measurements were made at 315 mμ.

ABSORPTION SPECTRUM OF CERIC ION

The absorption spectrum of a solution of ceric sulfate (1.92 × 10^{-5} *M*) in 1 *N* sulfuric acid, obtained with a Cary recording spectrophotometer (10.0-cm. cell), is shown in Figure 1. The maximum, which is fairly broad, occurs at approximately 320 mμ. The molar extinction coefficient is 5.58 × 10^3, permitting a photometric sensitivity of 0.025 microgram of cerium. The effect of acid strength on the location and height of the maximum is slight, over the range 0.1 to 6 *N* sulfuric acid.

DEVELOPMENT OF PROCEDURE

It was first attempted to determine cerium according to the procedure of Sandell (*8*), with the modification that the absorption was measured at 320 mμ, using a Beckman DU spectrophotometer with a 1-cm. silica cell. In this procedure, 10 ml. of solution are made 1 *N* in sulfuric acid, and 0.2 gram of ammonium

persulfate and 0.5 mg. of silver nitrate are added; the solution is boiled for 5 minutes, cooled, and diluted to 10 ml., and the transmittance is then measured. This procedure gave erratic results when the measurements were made at 320 mμ. Further study revealed two principal sources of error: absorption of light at 320 mμ by any persulfate remaining after boiling, and absorption by the nitrate ion formed by oxidation of the ammonium ion by persulfate. An absorption spectrum of 0.0010 *M* ammonium persulfate in 1 *N* sulfuric acid, measured in the 10-cm. cell of the Cary spectrophotometer, with 1 *N* sulfuric acid as the blank, is shown in Figure 2, *A*. The absorption increases continuously as the wave length is decreased; this is also seen from the curves of Figure 2, *B* and *C*, which were obtained with 0.010 and 0.10 *M* persulfate, respectively. An absorption spectrum of potassium nitrate is shown in Figure 3; the location of the maximum at 301.5 mμ has been established previously (*5*, *9*).

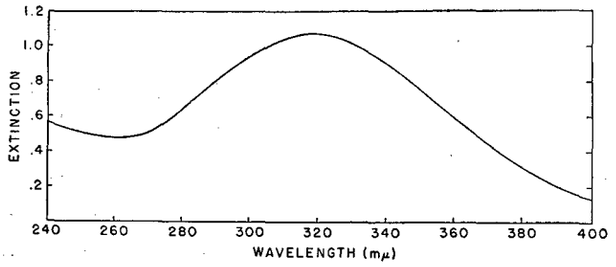

Figure 1. Absorption Spectrum of Cerium(IV) in 1 *N* Sulfuric Acid

Both of the above sources of error can be made negligible by using one tenth the amount of persulfate recommended by Sandell (*8*). While satisfactory results are obtained with this amount of ammonium persulfate, it is evident that potassium persulfate is to be preferred.

The solutions after oxidation are stable for 10 to 20 minutes, if water of good quality is used throughout; with water containing traces of organic matter, rapid fading has been found.

Table I. Recommended Procedure with Varying Amounts of Silver Nitrate and Potassium Persulfate

[All with 155 γ Ce(III), boiled 10 min.]

$AgNO_3$, Mg.	$K_2S_2O_8$, Mg.	Extinction (320 mμ)
0.50	24	0.640
0.12	24	0.648
None	24	0.598
2.50	24	0.660
0.50	6.0	0.640
0.50	96	0.666

Recommended Procedure. The amount of cerium taken should be between 0.04 and 0.20 mg. for maximum accuracy (although as little as 0.003 mg. can be detected), in 10 ml. of 1 *N* sulfuric acid in a 30-ml. beaker. To this solution are added 0.5 mg. of silver nitrate (0.2 ml. of a 0.25% solution) and 24 mg. of potassium persulfate (1.0 ml. of a 2.4% solution). A small silicon carbide chip is added to promote even boiling, the beaker is covered, and the solution is boiled for 5 to 10 minutes, with addition of water if necessary to maintain a volume of 6 to 10 ml. The beaker is then placed in cold (15°) water for 5 minutes, and the solution is transferred to a 10-ml. volumetric flask and made up to volume. The extinction is measured at 320 mμ in a silica cell, and the concentration of cerium is determined from a calibration curve.

RESULTS OBTAINED WITH RECOMMENDED PROCEDURE

Construction of Calibration Curve. To 5.00 ml. of ceric sulfate (0.0888 *N*) 0.1 *N* hydrogen peroxide was added until the solution was colorless. From this was prepared by successive dilutions a solution 1.11×10^{-4} *M* in cerous ion, in 1 *N* sulfuric acid; aliquots of this solution were treated by the above procedure, and the extinctions were measured in a 1-cm. cell with a Beckman DU spectrophotometer (hydrogen lamp, 0.34-mm. slit width).

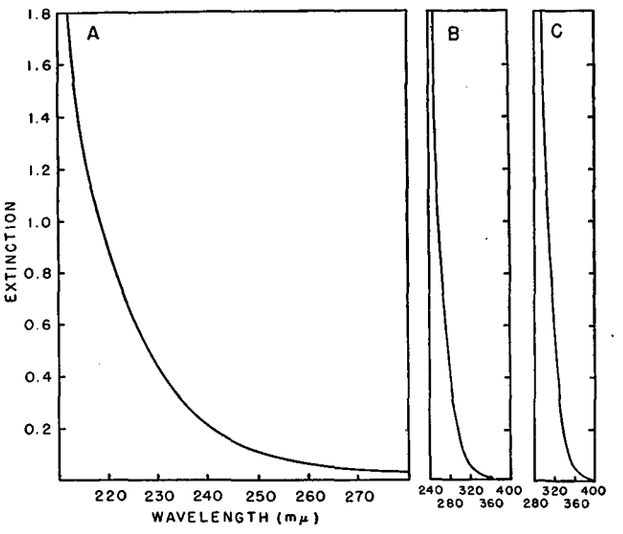

Figure 2. Absorption Spectra of Ammonium Persulfate in 1 *N* Sulfuric Acid

A. 0.001 *M* ammonium persulfate
B. 0.01 *M* ammonium persulfate
C. 0.1 *M* ammonium persulfate

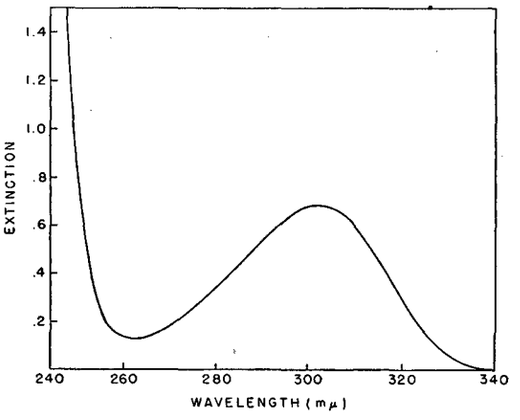

Figure 3. Absorption Spectrum of Potassium Nitrate (0.0101 *M*)

The results are shown in Figure 4. Beer's law is followed closely over the range studied; the extrapolated blank (0.003) is virtually negligible. Similar curves obtained at wave lengths other than 320 mμ are also shown in Figure 4; Beer's law is followed at these wave lengths also, although the sensitivity is, of course, not as great.

Variations in Recommended Procedure. TIME OF BOILING. Measured extinctions of solutions initially 1.11×10^{-4} *M* in cerous sulfate, oxidized according to the above procedure with various times of boiling, are shown in Figure 5. Satisfactory results are found with between 5 and 35 minutes of boiling.

AMOUNTS OF PERSULFATE AND SILVER NITRATE. The effects of variations in the amounts of these ingredients are shown in Table I.

Satisfactory results are obtained with the recommended amounts of silver and persulfate, or with as little as one fourth these amounts.

Table II. Oxidation of Ammonium Ion to Nitrate Ion in Absence of Cerium

(Solutions boiled 10 minutes, then made up to 10 ml.)

$K_2S_2O_8$, $M \times 10^2$	NH_4^+ $M \times 10^2$	$AgNO_3$, Mg.	H_2SO_4	Extinction (320 mμ)	NO_3^- Formed, $M \times 10^{2a}$
8.9[b]	..	..	..	0.054	..
8.9	17.8	0.50	..	0.079	1.8
8.9	17.8	0.50	1 *N*	0.017	0.2
8.9	17.8	..	1 *N*	0.008	0.0
0.89[c]	1.78[c]	0.50	..	0.023	0.4
0.89	1.78	0.50	1 *N*	0.005	0.1
0.89	1.78	..	1 *N*	0.004	0.0
0.89	200.	0.50	1 *N*	0.054	1.2
0.89	20.	0.50	1 *N*	0.005	0.1

[a] Calculated from extinction at 301 mμ, corrected for blank (boiled without ammonium sulfate).
[b] Unboiled; extinction due to persulfate.
[c] These amounts correspond to 20 mg. of ammonium persulfate (equivalent to 24 mg. of potassium persulfate).

Table III. Oxidation of Ammonium Ion to Nitrate Ion in Presence of Cerium

(All solutions 1 *N* in sulfuric acid, with 0.50 mg. of $AgNO_3$, 24 mg. of $K_2S_2O_8$, and 155 γ of cerous or ceric cerium. Solutions boiled 10 minutes, then made up to 10 ml.)

NH_4^+, $M \times 10^2$	Cerium	Extinction (320 mμ)	NO_3^- Formed, $M \times 10^{2a}$
..	Cerous	0.640	..
..	Ceric	0.643	..
200	Cerous	0.870	4.2
20	Cerous	0.665	0.4
2	Cerous	0.635	0.0
200	Ceric	0.837	3.2

[a] Calculated from extinction at 301 mμ, corrected for blank.

EFFECT OF AMMONIUM ION

Oxidation of ammonium ion to nitrogen and nitrate ion by persulfate in the presence of silver ion (at 25° C.) has been reported by Marshall and subsequent workers (*3, 4, 6, 7, 10*). Results obtained under the conditions of the present procedure are given in Tables II and III.

From the data of Table II it is seen that the persulfate-ammonia reaction leads to the formation of much more nitrate in initially neutral solution than in 1 *N* sulfuric acid; this effect of acid is in agreement with the observations of Marshall and Inglis (*7*). In 1 *N* acid, the nitrate formed from ammonium persulfate in the amount used by Sandell (*8*) is sufficient to give a significant extinction at 320 mμ, even in the absence of cerium.

With the recommended amount of potassium persulfate, and 2 *M* ammonium ion (added as ammonium sulfate), the amount of nitrate apparently formed is greater than would correspond stoichiometrically to the amount of persulfate taken. The significance of this is not clear at present.

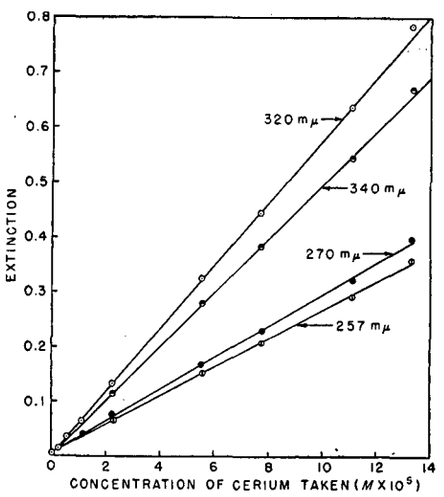

Figure 4. Extinction *vs.* Concentration of Cerium Taken in Recommended Procedure

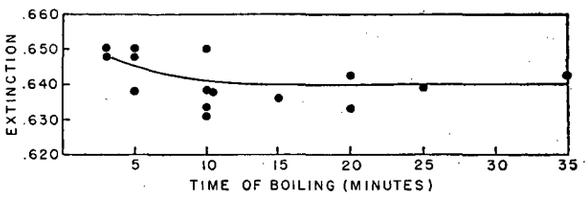

Figure 5. Extinction at 320 mμ *vs.* Time of Boiling

In the presence of 155 micrograms of cerium(III), the extent of formation of nitrate is severalfold higher than in the absence of cerium. Thus, with the recommended amount of persulfate, the initial presence of 0.2 *M* ammonium ion causes a significant error in the cerium determination, although the corresponding blank (Table II) is negligible. However, with 0.02 *M* ammonium ion, which is slightly greater than the amount of ammonium ion present in 20 mg. of ammonium persulfate (corresponding to the recommended amount of potassium persulfate), no error is found. When cerium is present initially in the quadrivalent rather than the trivalent state, the amount of nitrate formed (from 2 *M* ammonium ion) is considerably greater than in the absence of cerium; this indicates that the cerium acts here principally as a catalyst rather than by an induced reaction.

Table IV. Interference by Various Metals in Determination of Cerium

[All extinction coefficients are those of individual compounds; extinctions are those of indicated mixtures with 1.11×10^{-4} *M* cerium(III) carried through recommended procedure with 20 mg. of $(NH_4)_2S_2O_8$]

Compound Taken	Molar Extinction Coefficient, ε, Sq. Cm./Millimole			Amount Taken, *M*	Extinction	
	320 mμ	340 mμ	270 mμ		320 mμ	340 mμ
$Ce_2(SO_4)_3$ alone	4.83×10^3	4.11×10^3	2.26×10^3	1.11×10^{-4}	0.640	0.550
$CuSO_4 \cdot 5H_2O$	..	..	2.8×10^1	1.0×10^{-2}	0.639	0.550
				1.0×10^{-1}	0.676	0.556
$FeSO_4 \cdot 7H_2O$ or $Fe_2(SO_4)_3$[a]	6.3×10^2	3.3×10^2	5.8×10^2	2.0×10^{-5}	0.684	..
				1.0×10^{-4}	0.920	..
$K_2Cr_2O_7$	6.3×10^2	1.2×10^3	2.0×10^3	6.9×10^{-6}	0.642	..
				3.3×10^{-5}	0.653	..
				1.3×10^{-4}	0.707	..
$KMnO_4$	1.5×10^3	1.2×10^3	2.4×10^2	1.0×10^{-5}	0.634	..
				4.0×10^{-5}	0.641	..
NH_4VO_3	3.2×10^2	1.9×10^2	..	2.0×10^{-5}	0.639	..
				1.0×10^{-4}	0.661	..
$UO_2SO_4 \cdot 3H_2O$	1.2×10^2	2.3×10^1	1.0×10^3	1.0×10^{-5}	0.653	0.556
				1.0×10^{-4}	0.758	0.574
$Th(NO_3)_4 \cdot 4H_2O$	1.6×10^2	1.2×10^1	1.0×10^2	1.0×10^{-5}	0.636	0.552
				1.0×10^{-4}	0.660	0.563
KNO_3	3.0	..	1.9	1.0×10^{-2}	0.675	0.556
				1.0×10^{-1}	0.980	0.562

[a] Ferric sulfate taken for determination of extinction coefficient; ferrous sulfate used in analytical procedure.

EFFECT OF METAL IONS

A number of metal ions, of such a nature as would be expected either to occur with cerium in nature or to interfere with the cerium determination, have been studied according to the following plan. First, the absorption spectra of solutions of C.P. compounds in 1 *N* sulfuric acid were determined in the ultraviolet, with the Cary instrument (10-cm. cell). The extinction coefficients at 320 mμ were calculated, and on this basis various

amounts of each substance were taken, together with a known amount of cerium(III), for analysis according to the above procedure with ammonium persulfate (20 mg.) as reagent. With many metals it was evident from the absorption curves that the interference could be greatly reduced, at comparatively slight cost in sensitivity of cerium determination, by working at 340 rather than 320 mμ. The results are given in Table IV and Figures 6 and 7. Data for potassium nitrate are also included.

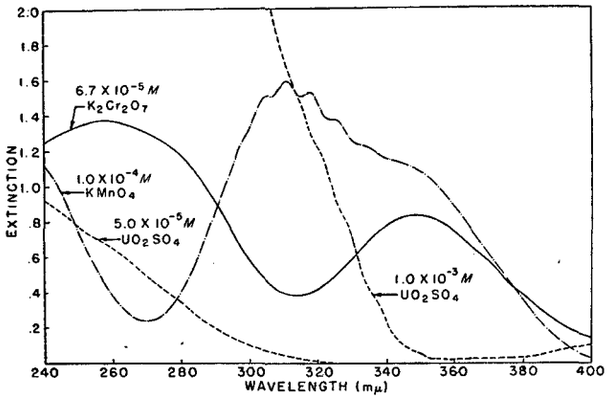

Figure 6. Absorption Spectra of Various Compounds in Ultraviolet

The molar extinction coefficients at 320 mμ of the majority of the compounds of Table IV are of the order of 10% of that of cerium(IV). For this reason separations must be made from a larger number of elements than if the color of the cerium(IV) is measured in the violet portion of the visible spectrum. On the other hand, the interference due to copper, particularly at 340 mμ, is so slight that it is possible to determine cerium accurately in a strong blue solution. The interference found with iron

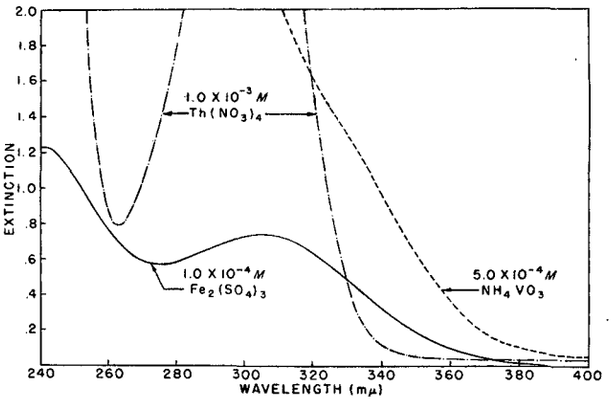

Figure 7. Absorption Spectra of Various Compounds in Ultraviolet

was higher than expected on the basis of its extinction coefficient; with manganese, on the other hand, less interference was found than expected.

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Spectrophotometric Determination of 8-Quinolinol

And Some of Its Halogenated Derivatives

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IN CONNECTION with a study of the mode of action of the iodine-containing 8-quinolinol compounds used for the treatment of amebiasis, it was desired to estimate quantities of the order of a fraction of a milligram of the drugs and their partially or completely dehalogenated derivatives. Other investigators (*1*, *2*, *4*, *7*, *10*, *12*) and the authors (*9*) have studied the absorption, distribution, and excretion of these iodinated compounds heretofore by determining the amount of iodine present in the biological materials by chemical or tracer techniques. However, it may not always be assumed that the iodine measured in this way is still bound to the 8-quinolinol nucleus, for it may have been removed by some metabolic process and subsequently transferred to some other moiety. Thus it becomes desirable to determine not only the iodine associated with the drug but also the quantity of 8-quinolinol present.

Published methods for the determination of 8-quinolinol depend on titration or gravimetric precipitation with bromine (*5*, *7*, *8*, *11*) and hence are not applicable to derivatives which are already substituted in the 5 and 7 positions. Fresenius (*6*) has described a spectrophotometric method for the determination of 5-chloro-7-iodo-8-quinolinol by measuring the color developed in glacial acetic acid in the presence of ferric iron. However, this method suffers because of its extreme sensitivity to the water content of the solvent. Grabbe (*7*) stated that a colorimetric method was employed for the estimation of small amounts of 8-quinolinol using ferric chloride in aqueous solution, but gave no details.

It was recognized that the intense green color of the complex formed by 8-quinolinols with ferric iron offered a sensitive and relatively specific method for their determination by spectrophotometry, provided a suitable solvent were available. It was found that methyl Cellosolve (2-methoxyethanol) was satisfactory for the purpose, as it is a good solvent for 8-quinolinol, 5-chloro-8-quinolinol, 5-chloro-7-iodo-8-quinolinol (vioform), 5,7-diiodo-8-quinolinol (diiodoquin), and their iron complexes. The color produced by the iron complexes in this solvent, which is not hygroscopic, is insensitive to small changes in water content. Methyl Cellosolve is not suitable for the determination of 8-quinolinol-5-sulfonic acid or 7-iodo-8-quinolinol-5-sulfonic acid.

In connection with a study of the mode of action of iodine-containing 8-quinolinol compounds (5,7-diiodo-8-quinolinol and 5-chloro-7-iodo-quinolinol) used for the treatment of amebiasis, it was desired to determine quantities of the order of a fraction of a milligram of the drugs and their partially or completely dehalogenated derivatives occurring in the urine of experimental animals. By employing the intense green color of the complex formed by 8-quinolinols with ferric iron and the selection of methyl Cellosolve (2-methoxyethanol) as a solvent, a spectrophotometric method was developed which is applicable to the determination of 8-quinolinol, 5-chloro-8-quinolinol, 5-chloro-7-iodo-8-quinolinol, and 5,7-diiodo-8-quinolinol in amounts of 0.05 to 0.50 mg. A procedure for the separation and determination of the components of mixtures of 8-quinolinol with any one of its halogenated derivatives and application to the analysis of urine containing these compounds in both the free and conjugated states is given. This method offers a simple rapid method for the estimation of 8-quinolinol compounds in urine and, with modifications, could be applied to many other types of materials.

because of their insolubility. In the procedure a considerable excess of iron over the theoretical ratio of 1 mole of iron to 3 moles of 8-quinolinol compound was used in order to ensure the maximum depth of color. It was found that acid concentrations below pH 1 destroyed or diminished the intensity of the color. When developed as described, the color was stable for several hours and showed little change over a 24-hour period when stored at room temperature in a stoppered container. The curves relating optical density, *D*, and wave length of incident light for the 8-quinolinol–iron complexes are given in Figure 1.

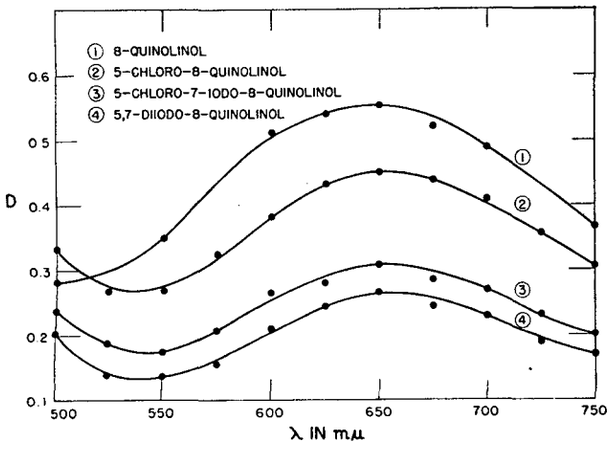

Figure 1. Optical Density *vs.* Wave Length of 8-Quinolinol–Iron Complexes

Concentration, 0.25 mg. of compound and 0.5 mg. of iron per 5 ml. of solution, 1-cm. cuvette

The maximum optical density was found to occur at 650 mμ for each of the compounds, indicating that halogen substituents have no measurable effect on the shade of the color.

METHOD

The reagents required are commercial grade methyl Cellosolve and an aqueous solution of ferric chloride containing 1 gram of ferric iron and 1 ml. of concentrated hydrochloric acid per liter.

The 8-quinolinol compound was dissolved in methyl Cellosolve by warming, if necessary, and diluted with the same solvent to a volume such that a 1-ml. aliquot contained 0.05 to 0.50 mg. of the compound. The aliquot was pipetted into a 10-ml. graduated cylinder, 0.5 ml. of the ferric chloride reagent was added, and the volume was adjusted to 5 ml. with methyl Cellosolve. The tube was stoppered and the contents were mixed by inverting the cylinder several times. The pH of this solution as measured with a glass electrode was 1.6 to 1.8 for all the 8-quinolinol compounds measured over the concentration range specified. The solution was transferred to a 1-cm. cuvette and the optical density measured at 650 mμ using as a blank a solution of 0.5 ml. of ferric chloride reagent diluted to 5 ml. with methyl Cellosolve.

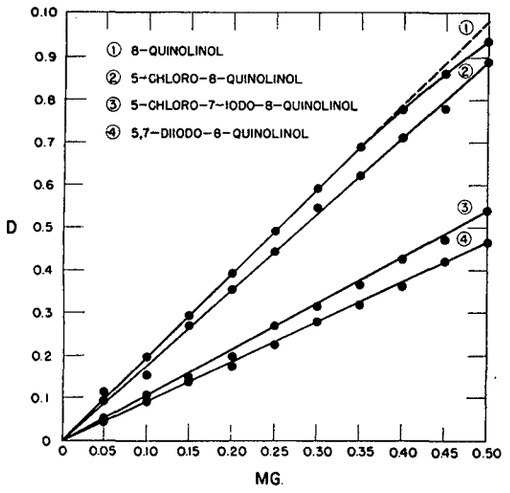

Figure 2. Optical Density *vs.* Concentration of 8-Quinolinol–Iron Complexes

1-cm. cuvette

Calibration curves (Figure 2) were prepared for each of the 8-quinolinol compounds having a satisfactory solubility in methyl Cellosolve. All were straight lines passing through the origin, with the exception of the curve for 8-quinolinol which deviates from a straight line at concentrations greater than 0.35 mg. In practice, *D* values greater than 0.6 were avoided by reducing the volume of the aliquot or increasing the dilution of the sample. This served to ensure a proper concentration of iron and to increase the precision of the measurement by using the lower portion of the logarithmic scale of the Beckman spectrophotometer.

An interesting relationship was noted when the calibration curves were plotted with the concentration expressed in micromoles instead of milligrams (Figure 3). The lines lie very close together, showing that the amount of color produced is primarily a function of the amount of the 8-quinolinol moiety present; the kind or number of the halogen substituents has only a minor effect. It was also found possible to analyze a mixture of 8-quinolinol with any one of its halogenated derivatives by measuring the optical density of the iron complex of the mixture, separating the halogenated compound, measuring its optical density, and subtracting the latter from the former to give the optical density equivalent to the amount of 8-quinolinol present in the mixture.

The 8-quinolinol was separated from its halogenated derivatives by pouring the methyl Cellosolve solution of the iron complex of the mixture after the optical density had been determined into 25 ml. of 1 *N* hydrochloric acid and extracting with five 5-ml. portions of ethylene dichloride. Under these conditions the

halogenated compound was quantitatively extracted, leaving the 8-quinolinol in the aqueous phase. The ethylene dichloride was evaporated at room temperature by a current of air and the residue was taken up in a small amount of methyl Cellosolve, 0.5 ml. of ferric chloride reagent was added, and the volume was adjusted to 5 ml. as before. The optical density of this solution subtracted from the optical density of the mixture is the optical density of the 8-quinolinol present in the mixture. If desired, the 8-quinolinol may be recovered from the aqueous hydrochloric acid solution by making it alkaline with sodium bicarbonate and extracting with several small portions of ethylene dichloride.

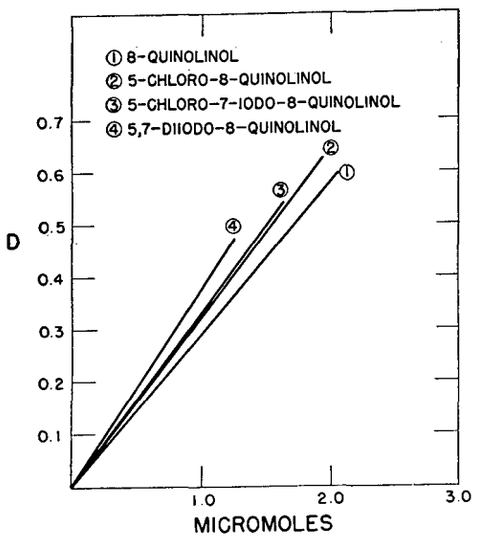

Figure 3. Optical Density *vs.* Concentration of 8-Quinolinol Complexes

1-cm. cuvette

A mixture of 0.100 mg. of 8-quinolinol and 0.175 mg. of 5,7-diiodo-8-quinolinol when analyzed as described gave *D* 0.372 for the mixture and *D* 0.167 for the diiodo compound. Subtracting, the optical density for 8-quinolinol was 0.205 or 0.102 mg. as read from the calibration curve, in good agreement with the theory.

Application to Urine Samples. The urine from rabbits receiving 8-quinolinol compounds orally was analyzed as follows:

The pH of the specimen was determined and, if it was below 7, sodium bicarbonate was added until a slightly alkaline reaction was obtained. A 50-ml. aliquot was extracted with five 10-ml. portions of ethylene dichloride, and the combined extracts were centrifuged to break the emulsions which usually formed. The clarified ethylene dichloride was filtered and evaporated to dryness with a current of air. The residue was dissolved in methyl Cellosolve and the determination carried out as described in the method. In the case of dosage with halogenated 8-quinolinols, it was also carried through the procedure for the determination of mixtures. These results gave the quantity of 8-quinolinol compounds present in the free state in the urine.

It has been shown by other investigators (*3, 7, 12, 13*) that 8-quinolinols occur in the urine as conjugates with sulfuric and glucuronic acids. These conjugates were not extracted by ethylene dichloride and gave no color with iron because the hydroxyl group was blocked. Hydrolysis of the urine samples with various concentrations of hydrochloric acid, both at room temperature and at 100° C. was unsatisfactory because of incomplete hydrolysis at low temperature and partial dehalogenation accompanied by an apparent disappearance of varying amounts of the 8-quinolinol nucleus at high temperatures and acid concentrations. These difficulties were circumvented by extracting the same sample used for the estimation of the free 8-quinolinol compounds with butanol. At least 8 extractions with 25 ml. of butanol each were required because of the relatively low solubility of the conjugates. It was found advisable to work up the last extract separately, in order to be sure that all the material had been extracted from the sample.

The butanol extract was centrifuged and filtered to break the emulsions and the solution was evaporated in vacuo, or by a current of air, at room temperature. The residue was taken up in 5 ml. of methyl Cellosolve, transferred to a 10-ml. volumetric flask, and diluted to volume with the same solvent. An aliquot of appropriate volume, as determined by trial, was transferred to a 10-ml. graduated cylinder and diluted to 4 ml., 0.5 ml. of ferric chloride reagent was added, and the volume was adjusted to 5 ml. with methyl Cellosolve.

No green color appeared on addition of the reagent, but within a few minutes a color change was apparent, as the acidic reagent caused hydrolysis of the conjugate with concurrent formation of the 8-quinolinol–iron complex. The rate of hydrolysis was followed by observing the change of optical density with time at 650 mμ. A typical curve, given in Figure 4, shows that the hydrolysis is virtually complete in 8 hours. For routine estimations it was unnecessary to follow the course of the hydrolysis. The solutions were allowed to stand at room temperature for 8 hours and then read; in fact, check determinations at 8 and 18 hours showed that there was no significant difference in the reading when the solutions were made up in the late afternoon and read the next morning. The hydrolyzed solution may also be analyzed for the presence of a mixture, as previously described.

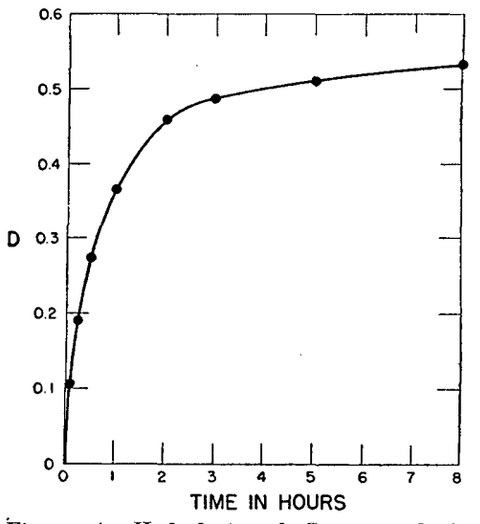

Figure 4. Hydrolysis of Conjugated 5-Chloro-7-iodo-8-quinolinol from Urine of Rabbits Receiving Vioform *per os*

Blank runs were made on urine from rabbits receiving the same diet but no 8-quinolinol compounds, and in no case was a green color produced by the residues from the extractions, thus eliminating any need for control blanks.

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Analysis of Binary and Ternary Mixtures

System Acetone-Chloroform–Methyl Isobutyl Ketone

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The limiting factor in the accuracy of phase equilibria data is often the method of analysis, especially for ternary systems. This work was undertaken to provide accurate methods of analysis for determining the binary and ternary vapor-liquid equilibria of the acetone-chloroform–methyl isobutyl ketone system. Accurate physical methods of analysis are presented for the binary and ternary mixtures of acetone, chloroform, and methyl isobutyl ketone. Analyses of ternary mixtures require measurement of any two of the three physical properties: refractive index, density, and viscosity; by measuring all three properties a weighted average of the points of intersection on a triangular composition grid can be used to determine the composition. The data presented can be used to determine the composition of mixtures of acetone, chloroform, and methyl isobutyl ketone with average accuracy of about 0.25 mole %.

THE determination of reliable vapor-liquid equilibria data and other phase equilibria data requires accurate analyses of the phases coexisting at equilibrium, and the results of the most careful experimental technique may be completely vitiated by inadequate analytical methods. Both chemical and physical methods of analysis have been used for this purpose, depending on the preferences of the investigator and the suitability of the method. If a chemical method of analysis is to be used, it is necessary to determine whether the methods suitable for the pure components are applicable to mixtures of two or more components. The most commonly used physical methods of analysis are refractive index, density, viscosity, boiling point or vapor pressure, optical density, and freezing point.

Table I. Physical Properties of Materials Used

	Boiling Point, °C.		Density, d_4^{25}		Refractive Index, n_D^{25}		Viscosity at 25° C., Centipoise	
	Observed	Literature	Observed	Literature	Observed	Literature	Observed	Literature
Acetone	56.13	56.10 (*15*)	0.7840	0.7840 (*16*) 0.7849 (*7*)	1.3560	1.3556 (*16*) 1.3566 (*14*)	0.299	0.3072 (*7*)
Chloroform	61.26	61.15 (*17*)	1.4787	1.4799 (*13, 17*)	1.4430	$1.4429^{25.4°}$ (*10*)	0.530	0.534 (*7*)
Methyl Isobutyl Ketone	115.91	115.8 (*3*)	0.7960	0.7960 (*9*)	1.3937	$1.3959^{20°}$ (*7*)	0.542	$0.5799^{20°}$ (*7*)

The analytical data presented in this paper serve as a means of determining the composition of mixtures of acetone, chloroform, and methyl isobutyl ketone. Although chemical methods of analysis were considered, no practical chemical method for differentiating acetone from methyl isobutyl ketone was known, and furthermore the determination of chloroform (*11*) was time-consuming and gave erratic results in the presence of acetone. Consequently, physical methods of analysis were investigated and after inspection of the differences of the physical properties of acetone, chloroform, and methyl isobutyl ketone, it was concluded that by the use of the three physical properties, refractive index, density, and viscosity, the optimum combination of accuracy and convenience would be had.

MATERIALS

The materials used in the present work were dried over Drierite and then distilled at a reflux ratio of 25 to 1 in a packed column containing the equivalent of 33 plates. The acetone was obtained as a 70% middle cut from Baker's chemically pure product. The methyl isobutyl ketone was also obtained as a 70% middle cut from a special product of Carbide and Carbon Chemicals Corp., which was reported 99.5% pure. The chloroform was obtained as a 60% cut from Baker's chemically pure product after rejecting a 30% forecut to ensure complete elimination of the ethyl alcohol stabilizer.

Acetone and methyl isobutyl ketone absorb moisture from the air. They were stored in tightly stoppered bottles and were distilled every 3 to 4 weeks. Chloroform decomposes in the presence of light and air, liberating hydrochloric acid and phosgene. It was, therefore, stored in tightly stoppered brown bottles, and fresh material was distilled every 2 weeks. Daily checks of the refractive indexes of all materials were made.

The physical properties of the materials used, as determined in this investigation, are given in Table I and are compared to what appear to be the most reliable literature values. Boiling points were also determined.

DETERMINATION OF PHYSICAL PROPERTIES

Refractive indexes, densities, and viscosities were determined at 25° ± 0.1° C. The temperature was controlled in a water bath equipped with an agitator and a circulating pump, by means of a mercury thermoregulator and an electric relay. The temperature was measured with a certified National Bureau of Standards thermometer.

Refractive Index. Refractive indexes were determined under sodium D light with a Spencer Abbe refractometer which has an acknowledged precision of ±0.0002 unit. However, through the use of a standardized procedure by a single individual, it appeared that somewhat better precision could be realized. The instrument was standardized by means of a glass crystal of known refractive index. Freshly distilled water was used as a secondary standard. In addition daily cross checks were made of the refractive indexes of acetone, chloroform, and methyl isobutyl ketone.

In order to minimize evaporation of the volatile solvents while transferring samples to the refractometer, all samples were chilled in ice water prior to loading. Constant controlled temperatures were established in approximately 1 minute. Tests showed that at this constant temperature samples in the refractometer did not change composition for 3 minutes. Therefore no error was introduced by allowing sufficient time for the chilled samples to attain the controlled temperature.

Density. Densities were determined by means of 10-ml. cap-type pycnometers (Ace Glass Co., Catalog 40-5475).

Chilled samples were transferred to the pycnometers which, after 20 minutes in the constant temperature bath, were removed; the overflows were quickly wiped; the pycnometers were chilled slightly, wiped thoroughly, allowed to come to room temperature, and weighed. The balance weights were calibrated against

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a standard 10-gram weight. Room temperature was always less than 25° C. so that the liquid never overflowed the capillary.

The pycnometers were calibrated with freshly distilled water having a density of 0.9971 gram per ml. at 25° C. Buoyancy

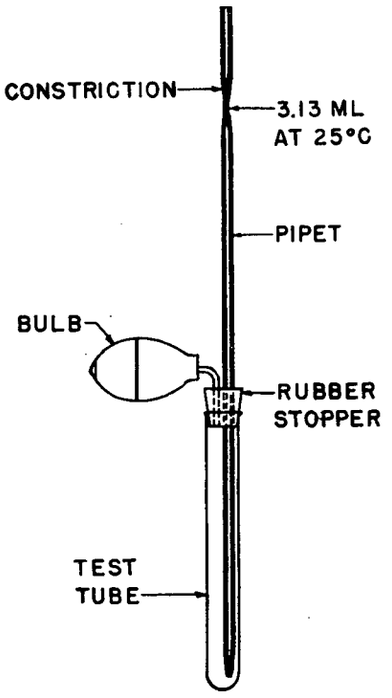

Figure 1. Loading Device for Viscometer

corrections were not applied, inasmuch as they were only significant in the fourth place and cancel out when the density-composition curve is calibrated on the same basis. Densities were determined with a precision of ±0.0002 gram per ml.

Viscosity. Viscosities were determined by means of Cannon-Fenske-Ostwald viscometers designed for liquids of 0.3 to 1.5 centistokes (*2*). A loading pipet, as shown in Figure 1, was

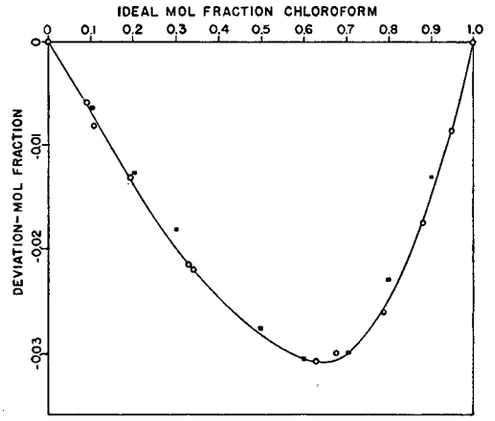

Figure 2. Refractive Index Deviation from Ideality, System Acetone-Chloroform at 25° C.

○ This investigation
■ Rosanoff and Easely (*10*)

made which delivered 3.13 ± 0.01 ml. at 25° C. The loading pipet gave better reproducibility and was more convenient than the usual method of loading Ostwald viscometers, which consists

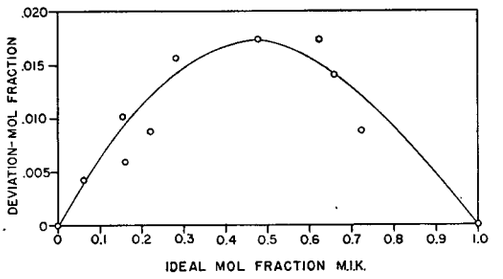

Figure 3. Viscosity Deviation from Ideality, System Acetone-Methyl Isobutyl Ketone at 25° C.

in drawing the liquid into the capillary to the etched mark by the application of suction.

A sample to be analyzed was placed in the constant temperature bath for 10 minutes. The loading pipet was then quickly inserted into the test tube, as shown in Figure 1. The pipet was filled by applying pressure to the bulb. The carefully meas-

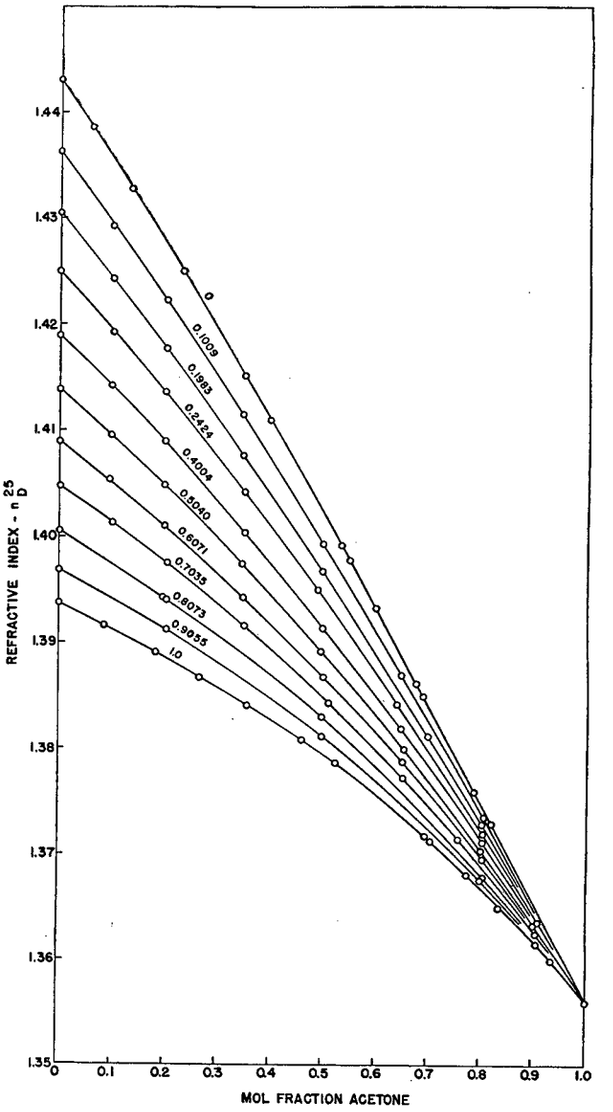

Figure 4. Ternary Refractive Index Calibration, System Acetone-Chloroform–Methyl Isobutyl Ketone at 25° C.

Parameter, mole fraction methyl isobutyl ketone in chloroform, acetone-free basis

ured sample was then transferred to the viscometer, in which the pipet fitted snugly, thereby minimizing evaporation losses. Both arms of the viscometer were stoppered for 5 minutes to allow the liquid to reach the bath temperature. By the application of air pressure, the liquid was forced into the capillary slightly above the etched mark. The viscosity in seconds was then measured in the usual manner by means of a precision electric timer having 0.1-second divisions.

Triplicate determinations within 0.2 second were usually obtained. This corresponds to a precision of 0.2 to 0.1% of the viscosity for the mixtures encountered in this investigation. Large deviations in the triplicate readings indicated foreign obstructions in the capillary; these determinations were repeated.

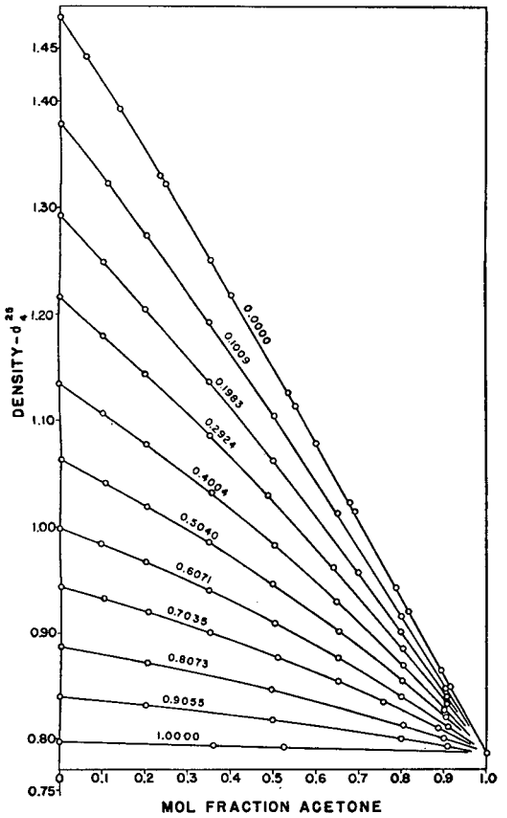

Figure 5. Ternary Density Calibration, System Acetone-Chloroform-Methyl Isobutyl Ketone at 25° C.

Parameter, mole fraction methyl isobutyl ketone in chloroform, acetone-free basis

In addition to obstructions in the capillary, other sources of error inherent in all Ostwald-type viscometers are loading errors and errors which result from a kinetic energy effect. The loading error in this investigation was approximately 0.01 ml. which corresponds to an error of 0.01% of the observed value, according to the equation given by Cannon and Fenske (*2*). Kinetic energy corrections for the viscometers used in this investigation were negligible. Therefore, determinations made with any two viscometers are related through the viscometer constants, K, of the instruments.

The viscometers were calibrated with freshly distilled water. The viscometer which was selected as standard had a K value of 0.003647 *centipoise* × cubic cm. per second × gram which was calculated from the equation:

$$K = \frac{\mu}{td} \qquad (1)$$

Table II. Physical Property Calibration of Binary Systems

Acetone Mole Fraction	Refractive Index, n_D^{25}	Density, d_4^{25}	Viscosity at 25° C., Seconds
	Acetone-Chloroform System		
0.9140	1.3640	0.8486	...
0.8932	...	0.8639	...
0.8185	1.3730	0.9194	...
0.7869	1.3760	0.9418	...
0.6892	1.3850	1.0137	...
0.6769	1.3862	1.0223	...
0.5979	1.3941	1.0775	...
0.5507	1.3978	1.1130	...
0.5327	1.3992	1.1255	...
0.4008	1.4110	1.2166	...
0.3517	1.4151	1.2499	...
0.2455	...	1.3219	...
0.2329	1.4250	1.3298	...
0.1359	1.4327	1.3929	...
0.0603	1.4385	1.4419	...
0.9630	...	...	104.5
0.9233	...	...	104.5
0.8196	...	...	106.0
0.6793	...	...	108.8
0.5520	...	...	111.7
0.4960	...	...	112.6
0.3737	...	...	112.8
0.2192	...	...	109.4
0.0555	...	...	101.3
	Acetone-Methyl Isobutyl Ketone System		
0.9323	1.3600	...	109.7
0.8350	1.3650	...	117.2
0.8344	1.3651	...	117.6
0.7731	1.3681	...	122.4
0.7067	1.3713	...	127.3
0.6923	1.3718	...	...
0.5275	1.3787	0.7906	...
0.5077	...	...	143.5
0.4615	1.3808	...	...
0.3590	1.3841	0.7924	155.7
0.3526	...	...	156.5
0.2689	1.3867	...	163.8
0.1842	1.3891	...	...
0.0872	1.3916	...	179.5
	Chloroform-Methyl Isobutyl Ketone System		
Chloroform Mole Fraction			
0.9657	1.4406	1.4451	
0.9429	1.4390	...	...
0.8979	1.4362	1.3786	...
0.8652	1.4340	...	...
0.8390	1.4325	1.3245	...
0.7985	1.4299	1.2892	...
0.7544	1.4273	1.2524	...
0.6975	1.4244	1.2067	...
0.6386	1.4207	...	...
0.6143	1.4197	1.1442	...
0.5473	1.4162	1.0971	...
0.5333	1.4157	1.0875	...
0.4321	1.4107	1.0220	...
0.3617	1.4073	0.9776	...
0.2445	1.4026	0.9144	...
0.1858	1.4003	0.8840	...
0.1420	1.3987	0.8621	...
0.0662	1.3960	0.8265	...
0.9291	...	...	109.3
0.8640	...	...	120.0
0.7252	...	...	141.4
0.6114	...	...	156.4
0.4970	...	...	167.6
0.3916	...	...	174.8
0.2352	...	...	181.1
0.1599	...	...	183.0
0.0813	...	...	184.6

where

μ = viscosity of water at 25° C. = 0.8939 centipoise.
d = density of water at 25° C. = 0.99707 gram per ml.
t = time of efflux = 245.8 seconds.

Viscosities determined in this investigation have been recorded in seconds, but can be converted to absolute units by means of the equation

$$\mu = 0.003647dt \qquad (2)$$

BINARY MIXTURES

Refractive indexes, densities, and viscosities of synthetic mixtures of the three binary systems, acetone-chloroform, acetone-methyl isobutyl ketone, and chloroform–methyl isobutyl ketone were determined. The data are given in Table II.

In order to improve the precision of the refractive index against composition curve for the acetone-chloroform system without ex-

cessive enlargement of the graph, a deviation plot similar to that used by Rosanoff and Easely (*10*) was constructed. The deviations of the actual composition from the ideal composition calculated for the given refractive index, assuming this property to be additive on a mole fraction basis, were plotted against the ideal composition in Figure 2. To use the deviation plot, the ideal composition is calculated arithmetically to the desired degree of precision and the small correction factor determined from the deviation plot is applied to obtain the actual composition. The refractive index calibration for the acetone-chloroform system agrees very well with that obtained by Rosanoff and Easely (*10*), whose data are also plotted in Figure 2.

A similar plot of the mole fraction deviations of the viscosities of the acetone–methyl isobutyl ketone system is shown in Figure 3. The deviations of this system from ideality are small, but

Table III. Physical Property Calibrations of Ternary Mixtures of System Acetone-Chloroform–Methyl Isobutyl Ketone

(Parameter, mole fraction methyl isobutyl ketone in chloroform, acetone-free basis)

Parameter	Acetone in Mixture, Mole Fraction	Density, d_4^{25}	Refractive Index, n_D^{25}	Viscosity at 25° C., Seconds
	0.9056	0.8477	1.3645	105.4
	0.8018	0.9154	1.3736	107.4
	0.6484	1.0125	1.3870	112.3
0.1009	0.4994	1.1034	1.3993	117.0
	0.3487	1.1918	1.4115	120.1
	0.2021	1.2732	1.4223	119.9
	0.1095	1.3223	1.4292	118.1
	0.000	1.3792	1.4362	114.1
	0.9104	0.8383	1.3640	105.7
	0.8018	0.9002	1.3729	108.9
	0.7010	0.9560	1.3812	112.8
0.1983	0.5003	1.0614	1.3967	120.8
	0.3495	1.1353	1.4076	126.6
	0.2027	1.2036	1.4177	130.4
	0.1017	1.2484	1.4242	131.0
	0.000	1.2926	1.4303	129.4
	0.9085	0.8324	1.3637	106.5
	0.8030	0.8853	1.3720	110.3
	0.6413	0.9612	1.3842	117.5
0.2924	0.4866	1.0299	1.3950	125.0
	0.3514	1.0846	1.4042	131.9
	0.1988	1.1434	1.4136	138.8
	0.1013	1.1787	1.4192	142.3
	0.000	1.2159	1.4248	144.0
	0.9024	0.8276	1.3637	107.5
	0.8036	0.8688	1.3712	111.7
	0.6497	0.9289	1.3819	119.5
0.4004	0.5011	0.9814	1.3914	128.3
	0.3532	1.0307	1.4003	137.5
	0.1992	1.0777	1.4090	147.0
	0.0993	1.1059	1.4142	153.2
	0.000	1.1335	1.4189	157.7
	0.9003	0.8209	1.3633	108.6
	0.8008	0.8549	1.3704	113.4
	0.6553	0.9007	1.3800	121.3
0.5040	0.4992	0.9451	1.3891	131.4
	0.3480	0.9843	1.3974	142.0
	0.2017	1.0190	1.4048	152.6
	0.0997	1.0412	1.4095	160.6
	0.000	1.0620	1.4138	167.7
	0.9089	0.8110	1.3626	108.7
	0.8013	0.8399	1.3696	114.5
	0.6535	0.8763	1.3788	123.9
0.6071	0.5038	0.9088	1.3868	134.2
	0.3500	0.9392	1.3942	145.8
	0.2002	0.9659	1.4010	157.9
	0.0953	0.9829	1.4053	166.8
	0.000	0.9980	1.4089	174.6
	0.8876	0.8092	1.3636	110.5
	0.7578	0.8348	1.3715	118.6
	0.5123	0.8764	1.3843	136.0
0.7035	0.6534	0.8537	1.3773	126.0
	0.3525	0.8997	1.3916	148.5
	0.2058	0.9188	1.3975	160.8
	0.1016	0.9310	1.4012	169.8
	0.000	0.9427	1.4047	179.0
	0.8986	0.7991	1.3624	110.7
	0.8055	0.8119	1.3679	116.8
0.8073	0.4989	0.8464	1.3830	139.7
	0.2060	0.8722	1.3940	164.1
	0.1979	...	1.3942	...
	0.000	0.8872	1.4005	182.2
0.9055	0.9080	0.7917	1.3616	...
	0.7984	0.7995	1.3677	118.7
	0.5007	0.8173	1.3811	142.4
	0.2036	0.8313	1.3912	166.5
	0.000	0.8390	1.3968	184.3

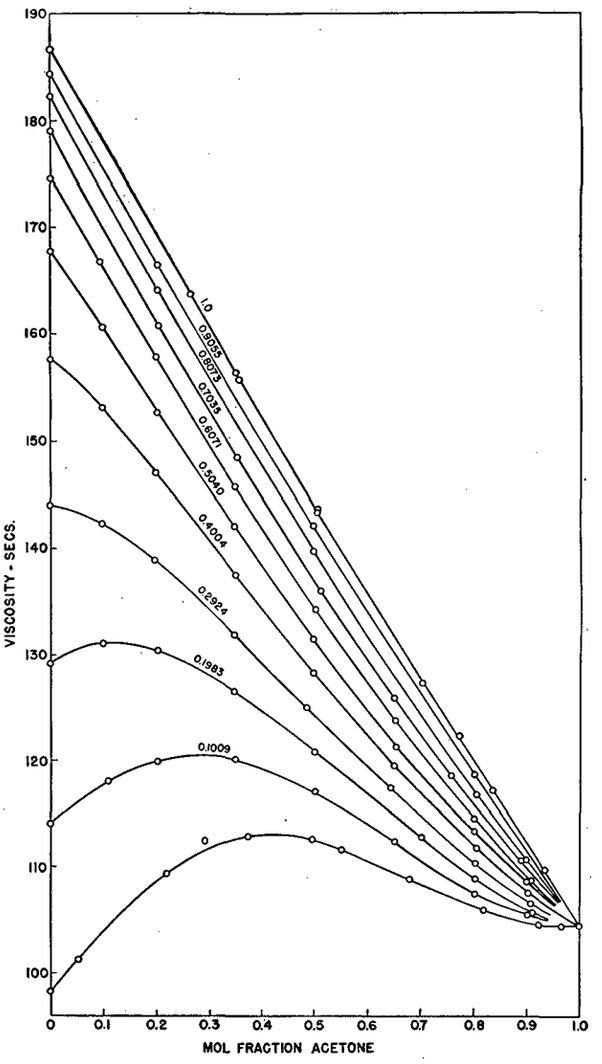

Figure 6. Ternary Viscosity Calibration, System Acetone-Chloroform–Methyl Isobutyl Ketone at 25° C.

Parameter, mole fraction methyl isobutyl ketone in chloroform, acetone-free basis

as Hatschek (*6*) has observed, "There are no mixtures which follow a linear law, in whatever way the concentration is expressed."

TERNARY MIXTURES

Various methods of analyzing ternary mixtures by physical means have been used (*1*, *4*, *5*, *12*). The physical properties and the best manner of determining and representing the calibration data are unique for each system.

The measurement of two physical properties of a ternary mixture is theoretically sufficient to determine its composition. In addition to the degree of precision of the measurements and the differences of the physical properties of the pure components, the ultimate analytical accuracy depends on the relation between the lines of constant values of the different physical properties on a triangular composition grid. The maximum accuracy is obtained when these contour lines cross each other at right angles.

In order to improve the reliability of the analyses of ternary mixtures of acetone-chloroform–methyl isobutyl ketone, three physical properties, refractive index, density, and viscosity, were used. The following advantages can thereby be gained over previous methods based on only two physical properties:

The weighted average of more than one point of intersection on the ternary composition grid can be used to determine the composition.

2. Large accidental analytical errors can be avoided.

In order to develop the physical-property diagram of the ternary system, different methyl isobutyl ketone–chloroform mixtures were prepared, to which increasing quantities of acetone were added. The refractive index, density, and viscosity of each resulting mixture were measured. Results are given in Table III.

A family of curves was obtained for each of the three physical properties against acetone concentration for constant values of the parameter, the mole fraction of methyl isobutyl ketone in the mixture on an acetone-free basis. These curves are shown in Figures 4, 5, and 6. Figure 7 was obtained by cross plotting these curves at constant values of the refractive index, density, and viscosity. This figure is primarily illustrative, and for the utilization of the present data a triangular diagram 18 inches along a side was used to present a readable grid containing lines of constant refractive index at intervals of 0.001 unit and lines of constant viscosity at intervals of 1 second. The lines of constant density were not drawn because they were straight lines and were readily obtainable by means of a straight edge passing through the proper terminal values.

This diagram represented the compositions of the original synthetic mixtures with a maximum deviation of 0.6 mole % and an average deviation of about 0.25 mole % for the lines of constant refractive index and viscosity. The average deviation of these data from the lines of constant density was about 0.1 mole %. These figures correspond approximately to the precision of the measurements and are a good indication of the accuracy of the analyses obtainable by this method.

The diagram was used to determine the composition of mixtures of acetone, chloroform, and methyl isobutyl ketone during an investigation of the ternary vapor-liquid equilibria for this system (*8*).

ACKNOWLEDGMENT

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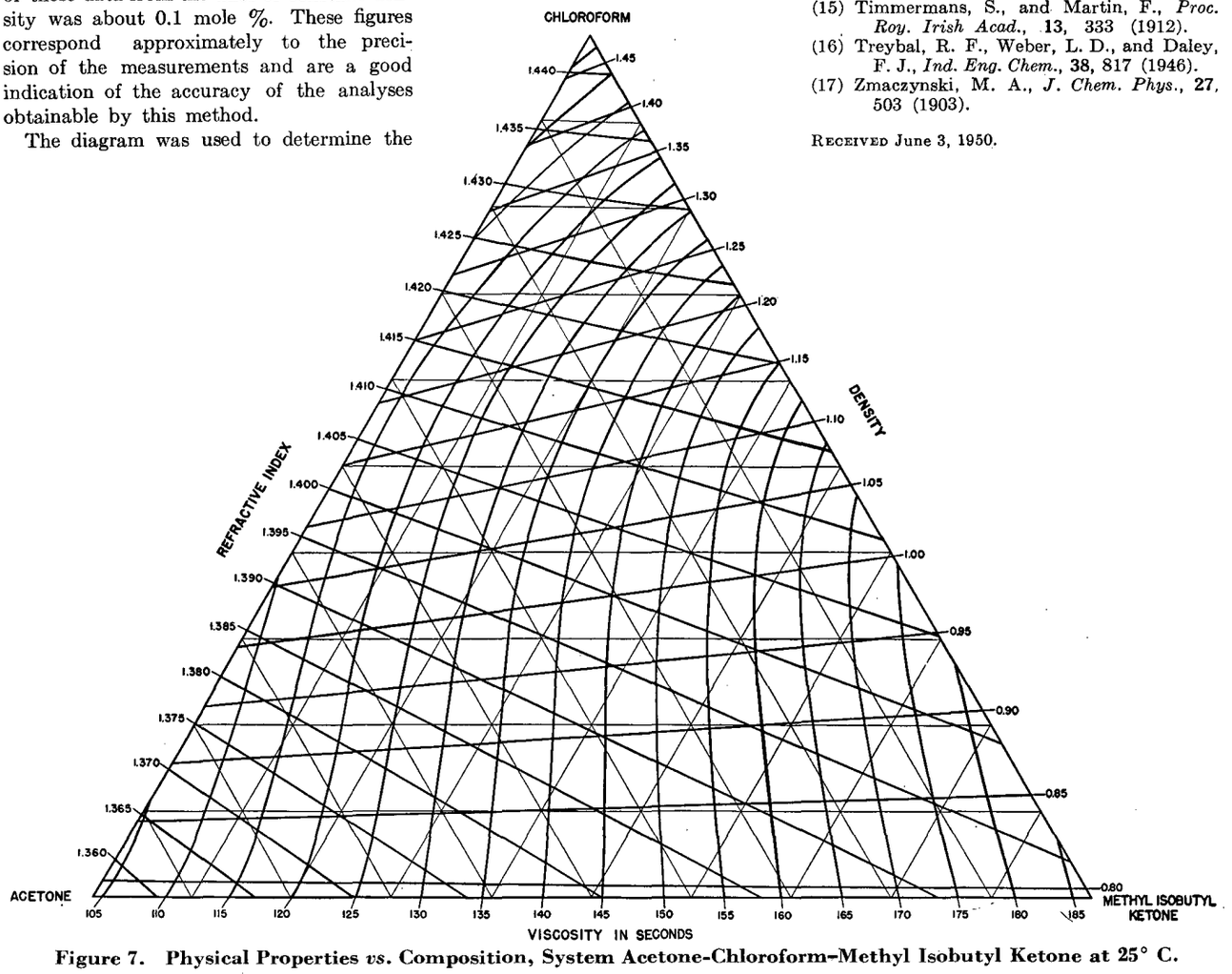

Figure 7. Physical Properties *vs.* Composition, System Acetone-Chloroform-Methyl Isobutyl Ketone at 25° C.

Base grid, mole per cent

Determination of Nitric Oxide and Nitrogen Tetroxide in Admixture

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In connection with a phase study of the system nitric oxide–nitrogen tetroxide, it was necessary to develop a precise procedure for the analysis of such mixtures. The data reported show that the procedure, as developed, is precise for nitric oxide, nitrogen tetroxide, and their admixtures. When a mixture of nitric oxide and nitrogen dioxide is absorbed in 95% sulfuric acid, nitric acid and nitrosyl sulfuric acid are formed. Total nitrogen in the solution is determined by means of a nitrometer and nitrosyl nitrogen by titration with a solution of potassium permanganate. A recovery of 99.8% was obtained for pure nitrogen tetroxide; 99.8% for pure nitric oxide; and for synthetic mixtures containing 4 to 10% nitric oxide the recoveries were 99.1% for nitric oxide, 99.8% for nitrogen tetroxide, and 99.5% for total nitrogen. A system, free from air and water, for absorbing the gases and including a special chamber for breaking ampoules containing the samples is described. The method is applicable to the assay of nitric oxide, nitrogen tetroxide, and mixtures containing up to 50 mole % of nitric oxide.

NUMEROUS methods for the determination of nitric oxide and nitrogen dioxide have been reported (*3, 8–11, 13*). The precisions that have been established in these methods are questionable because of the doubtful purity of the nitric oxide and nitrogen dioxide used.

Many investigators have used the nitrometer for determination (*2, 13*) of total nitrogen and titration with a solution of potassium permanganate (*12*) for "nitrosyl" nitrogen in solutions of nitric acid and nitrosyl sulfuric acid in concentrated sulfuric acid. Probably Milligan (*11*), who used these methods in determining nitric oxide and nitrogen dioxide in the quantitative determination of the reduction products of nitric acid, employed the best technique. However, he was interested in gaseous mixtures of nitrogen dioxide, nitric oxide, nitrous oxide, hydrazine, and other nitrogen-containing gases, and presented meager data on known mixtures of pure nitric oxide and pure nitrogen dioxide.

It was necessary to find a system with which the gases or their mixture would react quantitatively, and one which would be susceptible to a differential analysis that would give the purity of the gases or the composition of their mixture with a precision of 99.0% recovery or better; therefore, the "combined" methods of Milligan were thoroughly investigated with pure nitric oxide and pure nitrogen tetroxide.

The present report describes in detail the construction and operation of equipment applicable for the precise and convenient determination of nitric oxide and nitrogen tetroxide in admixture.

MATERIALS AND APPARATUS

Materials. Pure nitrogen tetroxide was prepared by the method of Whittaker, Sprague, and Skolnik (*16*), which is a modification of the method used by Giauque and Kemp (*5*). The purified material froze to a colorless solid, melting point −11.20° C., and contained 0.024 ± 0.01% water (method of Whitnack and Holford, *15*) and 0.15 ± 0.12% nitric oxide ($\bar{x}$ established after the precision of the method being reported was determined).

Pure nitric oxide was prepared by adding a saturated solution of sodium nitrite to a 35% solution of sulfuric acid saturated with iron(II) sulfate. The gas was purified by a modified version (*16*) of the method used by Johnston and Giauque (*7*). Upon infrared analysis, no other oxides of nitrogen were detected, but about 0.2% of inert material was indicated by the method being reported upon.

In the first absorber, 95 to 96% C.P. sulfuric acid was used; in the second absorber the same acid was used, and 2 ml. of 70% C.P. nitric acid were added for every 100 ml. of sulfuric acid.

Eastman Kodak reagent grade diphenylamine dissolved in C.P. concentrated sulfuric acid and a 35% solution of sulfuric acid saturated with iron(II) sulfate were used as indicators for possible loss of nitrogen oxides.

The "oxsorbent" of the Burrell Technical Supply Co. was used to remove oxygen from the nitrogen and indicating Drierite for removal of water.

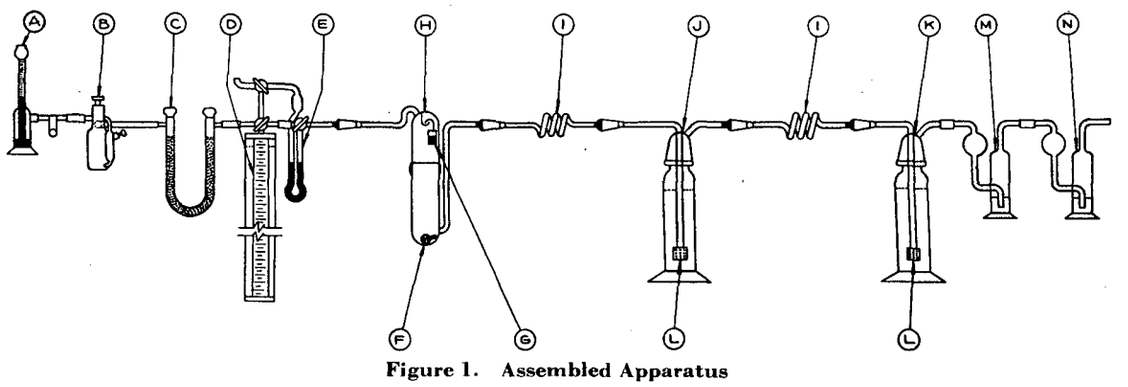

Figure 1. Assembled Apparatus

A. Mercury pressure gage
B. Oxsorbent vessel
C. U-tube (drying)
D. Gas buret
E. Pettersson compensation manometer
F. Breaking chamber
G. Plunger
H. ⅓ stopper with hook
I. Glass coils
J, K. Gas washing bottles
L. Fritted cylinders
M. Wash bottle (diphenylamine)
N. Wash bottle [iron(II) sulfate]

Dilute sulfuric acid for use in the nitrometer was prepared by diluting 100 ml. of 95 to 96% C.P. sulfuric acid with 243 ml. of distilled water. C.P. redistilled mercury was used in the nitrometer.

Potassium permanganate, 0.1 *N* and 0.03 to 0.05 *N*.

Iron(II) sulfate, 0.2 *N* containing 50 ml. of 96% sulfuric acid per liter.

Apparatus. Paired, calibrated 100-ml. and 25-ml. pipets.

A Du Pont nitrometer without the compensating tube.

The reaction system is shown in Figure 1. A mercury pressure gage is at *A*. *B* contains oxsorbent for removal of oxygen from the nitrogen and *C* is a U-tube filled with indicating Drierite. *D* is a 100-ml. water-jacketed buret graduated in 0.1 ml. for admission of nitric oxide. *E* is a Pettersson improved-form compensator manometer (*1*) with compensator tube and check valve for adjusting the nitric oxide in the buret to atmospheric pressure. The breaking chamber, *F*, is a 6 × 1 inch borosilicate glass tube fitted with a 5 × 1 inch hollow ⅋29/42 stopper. The glass plunger, *G*, is suspended from a glass hook inside the stopper and is filled with about 20 grams of iron filings. Glass coils, *I*, with ⅋10/30 joints are placed in the apparatus for flexibility. The absorption vessels, *J* and *K*, are Corning borosilicate glass tall-form gas washing bottles of 250-ml. capacity fitted with ⅋29/42 glass stoppers to which are attached coarse fritted cylinders 12 mm. in diameter. *M* is a small washing bottle containing the solution of diphenylamine, and *N* contains the iron(II) sulfate solution. All glass connections, except the absorbing vessel stoppers, are sealed with de Khotinsky cement.

PROCEDURE

Absorption of Gases. NITROGEN TETROXIDE OR NITRIC OXIDE–NITROGEN TETROXIDE MIXTURES. A small ampoule, containing 0.5 to 1.0 gram of sample, is weighed and placed in the breaking chamber. The ⅋ stopper, *H*, holding the plunger is then put in place and sealed with DeKhotinsky cement. The system including the breaking chamber is swept out with a stream of dry nitrogen for 0.5 hour. The first absorption vessel is filled with 200 ml. of 95% sulfuric acid and the second with 200 ml. of the sulfuric acid containing a small amount of nitric acid. The vessels are then connected to the apparatus, the connections are sealed with de Khotinsky cement, and the entire system is freed of air and water vapor by a stream of dry nitrogen. Complete removal can be assumed after 4 hours. A Dewar flask, containing a dry ice–alcohol mixture, is placed around the lower half of the breaking chamber, to lower the rate of transfer of the gases after the ampoule is broken. The plunger is removed from the glass hook, using two bar magnets, and allowed to fall, breaking the ampoule.

When the pressure in the system lowers sufficiently, dry nitrogen is admitted so that the gases can be completely transferred from the breaking chamber to the absorption vessels. (The dry ice–alcohol bath cannot be kept around the breaking chamber continuously, or the nitric oxide–nitrogen tetroxide mixture will freeze.) As the pressure within the system falls, the pressure of the entering nitrogen is increased in order to maintain a constant rate of flow through the system. Absorption of the oxides may be assumed to be complete 1 hour after the last visible trace of nitrogen dioxide has left the breaking chamber. If the indicating solutions at the end of the system show loss of nitrogen oxides, the determination should be discarded. A slow rate of absorption will prevent loss of the oxides of nitrogen. Usually crystals of nitrosyl sulfuric acid form on the inside of the entrance tube of the first absorber. These are dissolved in the acid medium near the end of the operation by shutting off the flow of nitrogen and alternately heating and then cooling the breaking chamber with the dry ice–alcohol mixture.

NITRIC OXIDE. The procedure is the same as for nitric oxide–nitrogen tetroxide mixtures, except that the sulfuric acid containing a small amount of nitric acid is used in both absorbers. The absorption media in both vessels are analyzed only for "nitrosyl" nitrogen, using the more dilute solution of potassium permanganate.

Analysis of Absorption Media. TOTAL NITROGEN (First absorber). A 25-ml. pipet is used to deliver an aliquot of the absorption medium from the first absorption vessel into the nitrometer cup. The solution in the cup is then drawn into the reaction bulb without allowing air to enter the vessel. The cup is washed with two 2.5-ml. portions of the diluted sulfuric acid, and the mixture is vigorously shaken. The shaking should be continued for a minute after action seems to have ceased. The reaction vessel is connected to the buret and the nitric oxide liberated by the reaction is carefully measured. The total volume of measured gas is then corrected to standard temperature and pressure.

The gas produced by the reaction in the nitrometer is nitric oxide. Tower (*14*) has shown that 0.0193 ml. of nitric oxide will dissolve in 1 ml. of 90% sulfuric acid at 18° C. and 760 mm. of nitric oxide pressure. The correction for the solubility of nitric oxide, then, is 0.58 ml. when 25-ml. aliquots and 5 ml. of wash acid are used. The difference between this value at 18° C. and at 0° C. (S.T.P.) is negligible. The true volume of nitric oxide produced from the 25-ml. aliquot of sample is then the measured volume corrected to S.T.P. plus the solubility correction factor of 0.58 ml.

Table I. Purity of Nitrogen Tetroxide

Added *Mg.*	Found *Mg.*	Recovered %
794.1	792.6	99.8
1059.7	1058.1	99.8
892.7	892.7	100.0
1029.8	1029.2	99.9
887.7	885.0	99.7
941.3	941.3	100.0
1046.8	1043.7	99.7

Average, $\bar{x}$ = 99.8%
Standard deviation, S = 0.13% absolute
Standard deviation of mean (of 7), S_m = 0.049% absolute
Confidence range, 99.8 ± 0.12%
$\bar{x}$ − true value = −0.2 ± 0.05% absolute

NITROGEN AS NITROSYL SULFURIC ACID (First absorber). The amount of nitrosyl nitrogen is determined by titration with a solution of potassium permanganate. A 25-ml. aliquot of the absorption medium in the first absorption vessel is added to a measured excess volume of standard 0.1 *N* (0.03 to 0.05 *N* when determining pure nitric oxide) potassium permanganate (ca. 40 ml.) that has been diluted to about 500 ml. with water. The aliquot should be added slowly with constant stirring, and the tip of the pipet must be kept just under the surface of the liquid to prevent any loss of nitrosyl sulfuric acid through air oxidation. These conditions were found best for sharpness of the end point and prevented loss of nitric oxide, which would occur if the acid were diluted and titrated directly. The iron(II) sulfate solution is added from a buret until the permanganate that remains is used up and an excess of iron(II) sulfate is present. The excess iron(II) sulfate is then back-titrated with permanganate. A blank determination on a 25-ml. portion of the sulfuric acid (95 to 96%) is made in the same manner. The difference between the sample and blank represents the permanganate used by the sample.

NITROGEN AS NITROSYL SULFURIC ACID (second absorber). Aliquots of the absorption medium (25 ml.) are analyzed as in the first absorber.

Calculations. Based upon the following equations representing the reactions that occur in the absorbers, the percentages of nitric oxide and nitrogen tetroxide are calculated according to Milligan (*11*).

$$2NO_2 + H_2SO_4 \longrightarrow SO_2(OH)(NO_2) + HNO_3 \quad (1)$$

$$NO + NO_2 \rightleftharpoons N_2O_3 \quad (2)$$

$$N_2O_3 + 2H_2SO_4 \longrightarrow 2SO_2(OH)(NO_2) + H_2O \quad (3)$$

$$2NO + HNO_3 + 3H_2SO_4 \longrightarrow 3SO_2(OH)(NO_2) + 2H_2O \text{ (alternate for Equations 2 and 3)} \quad (4)$$

Let
A = total nitrogen (first absorber)
B = "nitrosyl" nitrogen (first absorber)
X = nitrogen in NO_2 absorbed
Y = nitrogen in N_2O_3 absorbed (Equations 2 and 3)
Z = "nitrosyl" nitrogen (second absorber, Equation 4)

Then $A = X + Y$ and $B = (0.5)X + Y$

Therefore

$$NO_2 \text{ absorbed} = 2\frac{NO_2}{N}X + \frac{NO_2}{2N}Y = (3.285)X + (1.642)Y$$

$$NO \text{ absorbed} = \frac{NO}{2N}Y = (1.071)Y \text{ (1st absorber)}$$

$$NO \text{ absorbed} = \frac{2NO}{3N}Z = (1.428)Z \text{ (2nd absorber)}$$

DATA

To ascertain the precision and reliability of the individual methods, several experiments were made with pure nitrogen tetroxide and pure nitric oxide. The results in Table I indicate

Table II. Purity of Nitric Oxide (6)

Added	Found	Recovered
Mg.	*Mg.*	%
59.8	59.5	99.5
53.4	53.5	100.2
56.8	56.5	99.5
54.4	54.2	99.6
86.0	85.4	99.3
85.4	85.6	100.2

Average, $\bar{x}$ = 99.7%
Standard deviation, S = 0.39% absolute
Standard deviation of mean (of 6), S_m = 0.16% absolute
Confidence range, 99.7 ± 0.41%
$\bar{x}$ − true value = −0.3 ± 0.16% absolute

Table III. Recovery of Nitric Oxide and Nitrogen Tetroxide from Known Mixtures

	Added	Found	Recovery
	Mg.	*Mg.*	%
NO	100.8	99.4	98.6
NO_2	1011.1	1007.0	99.6
N	354.7	352.8	99.5
NO	110.4	109.6	99.3
NO_2	1112.8	1105.6	99.4
N	390.1	387.6	99.4
NO	92.0	90.7	98.6
NO_2	985.6	985.2	100.0
N	342.9	342.1	99.8
NO	49.0	48.7	99.4
NO_2	1043.3	1038.2	99.5
N	340.4	338.6	99.5
NO	51.1	50.9	99.6
NO_2	1131.5	1126.1	99.5
N	368.2	366.4	99.5

	NO	NO_2	N
	%	%	%
Av., $\bar{x}$	99.1	99.8	99.5
Standard deviation, S	0.42 (abs.)	0.32 (abs.)	0.16 (abs.)
Standard deviation of mean (of 5)	0.19 (abs.)	0.085 (abs.)	0.07 (abs.)
Confidence range	99.1 ± 0.53	99.8 ± 0.39	99.5 ± 0.19

that the determination of total nitrogen by means of the nitrometer has a precision, shown by a standard deviation obtained from seven values, of about 1.5 parts per thousand with pure nitrogen tetroxide samples. The results in Table II indicate that the determination of nitrogen as nitrosyl sulfuric acid by titration with potassium permanganate has a precision, shown by a standard deviation obtained from six values, of about 4 parts per thousand with pure nitric oxide. (The same precision was established for the determination with potassium permanganate of the nitrogen tetroxide samples in Table I.)

Table IV. Nitric Oxide in Purified Nitrogen Tetroxide

Nitrogen Tetroxide Added	Nitric Oxide Found	
Mg.	*Mg.*	%
794.1	1.5	0.19
1059.7	1.6	0.15
892.7	0.0	0.00
941.3	0.0	0.00
1029.8	0.6	0.06
1046.8	3.1	0.30
887.7	2.8	0.32

Average, $\bar{x}$ = 0.15%
Standard deviation, S = 0.13% abs.
Standard deviation of mean (of 7), S_m = 0.05%
Confidence range (fiducial limits) $\bar{x} \pm tS_m$ = 0.15% ± 0.12%

The precision and reliability of the "combined" method for nitric oxide and nitrogen tetroxide were established with synthetic mixtures of pure nitric oxide and pure nitrogen tetroxide. The results in Table III indicate that the method has a precision, shown by a standard deviation obtained from five values, of about 4 parts per thousand for nitric oxide, 3 parts per thousand for nitrogen tetroxide, and 1.5 parts per thousand for total nitrogen. On the basis of these results a confidence range of 99.5 ± 0.19% may be expected for total nitrogen recovery on samples of nitric oxide and nitrogen tetroxide in admixture.

Several samples of purified nitrogen tetroxide were analyzed for nitric oxide by the method described. The results in Table IV indicated an average value of 0.15% nitric oxide. However, a confidence range of 0.15 ± 0.12% indicates that this amount of nitric oxide is probably meaningless.

Laboratory samples of liquid mixtures of pure nitric oxide and pure nitrogen tetroxide were analyzed by the method described. The results in Table V establish a range of 2 to 25% nitric oxide in the liquid mixtures and indicate total recovery percentages within the precision established for the method.

The indexes of precision used in the statistical analysis of data presented in the tables are as follows:

Standard deviation (estimate) $S = \sqrt{\Sigma(x - \bar{x})^2/(n - 1)}$
Standard deviation of mean of n, $S_m = S/\sqrt{n}$
Confidence range (fiducial limits) $= \bar{x} \pm tS_m$
In the above
$\bar{x}$ = mean of n observations of x
t = Student's "t" (4) for the significance level desired and $n - 1$ degrees of freedom. For the 1 in 20 significance level and means of 7 (6 degrees of freedom) t is 2.45.

DISCUSSION

In the early phase of the development work with nitrogen tetroxide, a few experiments were made using but one absorber. As the results obtained were unsatisfactory, two absorbers were used in the rest of the work and the indicating solutions were used at the end of the system. Very careful control of the rate of transfer of the gases from the breaking chamber to the absorbers is necessary, and for this reason the dry ice–alcohol bath is used. With improvement in technique in handling the gases, only rarely was any nitric oxide found in the second absorber.

The purity established for the nitric oxide and nitrogen tetroxide was used in determining the precision of the method for the synthetic mixtures. In this process a definite amount of nitric oxide was slowly added from the buret to the system after the ampoule containing nitrogen tetroxide had been broken. A dry ice–alcohol bath was used to slow the rate of transfer of the gases over into the absorbers.

The authors found, in agreement with Milligan (*11*), that the use of 88% sulfuric acid for the reaction in the nitrometer gave satisfactory results.

Table V. Analyses of Laboratory Samples of Mixed Nitric Oxide and Nitrogen Tetroxide

Sample	Nitric Oxide Found	Nitrogen Tetroxide Found	Recovery ($NO + N_2O_4$)
Grams	%	%	%
1.1256	2.2	97.3	99.5
0.5861	8.8	89.4	98.2
0.3103	12.3	88.1	100.4
0.3356	15.4	84.8	100.2
0.3334	20.7	78.3	99.0
0.1579	24.9	75.2	100.1

For ease in detecting the end point of the titration, when using 0.1 N solutions of potassium permanganate, dilution with 500 ml. of water was most satisfactory. With the more dilute potassium permanganate solution only 200 ml. of water were used.

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Determination of Citric and *d*-Isocitric Acids

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d-Isocitric acid is one of the components of the series of enzymatic reactions generally referred to as the tricarboxylic acid cycle of Krebs, a mechanism that is frequently advanced as the explanation of respiration in living cells. The substance is thus probably widely, if not universally, distributed, and it is known to occur in substantial quantities in the leaves of certain plants. The Krebs and Eggleston method of determining isocitric acid depends upon the use of aconitase, which converts a definite proportion of the isocitric acid to citric acid, which is in turn oxidized and brominated to pentabromoacetone. It has now been found that if metaphosphoric acid is present during the oxidation of citric acid, the conversion can be made essentially quantitative and that considerable latitude is then permissible in the conditions, such as acidity, time, temperature, etc., which it has hitherto been necessary to control with care. A marked improvement in the pentabromoacetone method of determining citric acid and, accordingly, of isocitric acid has thus been effected, the precision being now of the order of 1 to 2%. The way is thus opened for the study of the metabolism of isocitric acid in plants.

SEVERAL fundamentally different methods for the determination of *d*-isocitric acid have been described (*6, 8, 10*). Of these, the method of Krebs and Eggleston, which depends upon the conversion of *d*-isocitric acid to citric acid by the enzyme aconitase, has advantages that commend it for use in the study of organic acid metabolism in plant tissues. Under the action of this enzyme, an equilibrium is established such that the ratio of citric–*cis*-aconitic–*d*-isocitric acids at 38° C. is 89.5–3.9–6.6% (*3, 5*). Accordingly, it suffices to determine citric acid before and after treatment of samples with the enzyme; from the increase in citric acid, the sum of isocitric and *cis*-aconitic acids can be calculated. Because *cis*-aconitic acid does not appear to be present in significant quantities in the plant tissues with which the authors have been concerned, the method may be used for the determination of isocitric acid in them.

In the course of a study of the Krebs and Eggleston (*6*) method for isocitric acid, a number of observations have been made that throw light upon certain of the hitherto imperfectly understood details of the pentabromoacetone procedure for the determination of citric acid. As a result, modifications have been made of the method of Pucher, Vickery, and Leavenworth (*15*) of determining citric acid which increase both its convenience and accuracy.

That the earlier titrimetric (*12, 15*) and colorimetric (*14*) methods leave something to be desired is obvious from a number of papers that describe modifications of one or another of the procedures (*4, 6, 9, 17, 19–21*). The critical points appear to be the acidity at which the oxidation is conducted, the rate of addition of the permanganate, the temperature, the time allowed for the oxidation, and the precise nature of the oxidizing agent. Under the conditions described by Pucher, Vickery, and Leavenworth, the yield of pentabromoacetone was close to 90% of theory. Goldberg and Bernheim (*4*) showed that the yield is a function of the acidity at which the oxidation is carried out, ranging from about 90% in 1 *N* sulfuric acid to about 105%, owing to the formation of some hexabromoacetone, in 9 *N* acid, but diminishing at still higher acidities. Approximately quantitative results were obtained in 4 to 5 *N* acid. Similar observations were made by Taussky and Shorr (*19*) as well as in the present work.

It was noted, in the course of oxidations of solutions of citric acid carried out after treatment of mixtures of citric and isocitric acids with aconitase and subsequent deproteinization with metaphosphoric acid, that the recoveries of citric acid as pentabromoacetone were invariably close to 98% of theory. This was true even when the acidity was 1 *N* or somewhat less, conditions that led to only 89% recovery with the usual oxidation procedure before treatment with aconitase. Furthermore, the appearance of these solutions during oxidation differed, inasmuch as there was no development of turbidity nor formation of a brownish color owing to the separation of manganese dioxide; the color of the clear solution remained that of permanganate. More careful observation of the oxidation at high acidities showed that the solutions also remained clear at or above an acidity of about 4 *N*.

The difference in behavior between solutions before and after the treatment with aconitase was traced to the presence of metaphosphoric acid. This reagent appears to form complex compounds such that, if it is present, no precipitation of manganese dioxide takes place. The system is therefore homogeneous during the oxidation reaction and the end result is the formation of pentabromoacetone in essentially quantitative yield. The desirability of a homogeneous system is also illustrated by the behavior of solutions oxidized at high acidity, which likewise give high yields.

Accordingly, the conditions under which the oxidation and bromination are carried out were modified by adding a sufficient amount of metaphosphoric acid to prevent the separation of manganese dioxide. It was then found that a system had been developed that was no longer sensitive to a number of the conditions, the rigid control of which had been regarded as essential by some workers with this method. As is clear from Table I, there was very little effect if the rate of addition of the permanganate were varied over a wide range; on the contrary, it seemed desirable to

add an excess all at once from a rapid pipet, and stirring was not necessary. Constant recoveries of 98% were obtained at acidities from 1 *N* to 2 *N*, so that only approximate adjustment of the acidity is essential; at higher acidities, the formation of hexabromoacetone appears to be increasingly stimulated. The time required for the reaction may be varied from 3 to 15 minutes, the quantity of citric acid present is immaterial within reasonable limits, and the temperature may be at any convenient point below 20° C., although it should not be allowed to exceed this more than a degree or so. On the other hand, in the absence of metaphosphoric acid, the extent of the reaction is greatly affected by the rate of addition of the permanganate.

Table I. Yield of Pentabromoacetone from Citric Acid

(After oxidation with permanganate in presence of bromide, expressed as percentage of theory[a])

Variable Studied		No. of Detns.	Yield, %
Time of addition of 5 ml. of $KMnO_4$, seconds (solution stirred, metaphosphoric acid absent)	5	18	88.8
	45	25	94.1
	90	4	99.5
Volume of 20% metaphosphoric acid, ml. (stirring not necessary)	0	18	88.8
	0.4	2	96.2
	0.6	2	97.4
	1.0	4	98.2
	2.0	2	98.4
Time of addition of 5 ml. $KMnO_4$, seconds (1.0 ml. of metaphosphoric acid added)	7	42	98.0
	30	2	97.4
	45	2	97.8
	60	2	97.6
	90	2	97.6
	600	2	98.2
Time allowed for oxidation to proceed, minutes (metaphosphoric acid added)	1	2	94.8
	3	1	98.8
	5	2	98.7
	10	3	98.3
	15	2	98.3
	30	2	94.5
	60	2	90.2
Concentration of H_2SO_4 during oxidation, normality (metaphosphoric acid added)	0.5	2	96.4
	1.0	2	98.0
	1.5	2	98.0
	2.0	2	98.2
	3.0	1	100.6
	5.0	2	102.2
	8.0	2	106.8
	10.0	2	103.2
	14.0	2	64.0
Quantity of citric acid taken, mg. (metaphosphoric acid added)	0.5	4	98.2
	1	10	97.9
	2	24	98.0
	3	4	98.3
Temperature during oxidation, ° C.	3–4	1	97.6
	18–19	2	97.8
	33–35	2	89.8
	45–50	2	75.5

[a] One variable was examined at a time in each group of tests. Except in first group, oxidation mixture was not stirred after addition of permanganate.

The other important modification of the original titrimetric method is the use of the Sendroy silver iodate procedure (*18*) for the determination of the bromide liberated by the sodium sulfide. (For the suggestion of this technique, the authors are indebted to the late G. W. Pucher, who, at the time of his death, was engaged in preliminary tests of the applicability of the Sendroy method.) This change contributes greatly to the convenience and precision of the titration and makes it possible to extend the scale of the method to the microgram range if desired.

REAGENTS

Sulfuric acid, 18 *N* (*15*); potassium permanganate, 1.5 *N* (*15*); potassium bromide, 1 *M* (*15*); phosphoric acid, 0.85 *M* and 0.085 *M* (*18*).

Phosphoric acid, 2.0 *M*. 136 ml. of concentrated phosphoric acid (specific gravity 1.7) made to 1000 ml. with water.

Nitric acid, 1.0 *N*. 32 ml. of concentrated nitric acid (specific gravity 1.42) made to 500 ml.; 0.05 *N* obtained by twentyfold dilution.

Sodium thiosulfate, 0.1 *N* stock solution. 24.82 grams of pentahydrate made to 1000 ml. with water and 5 ml. of chloroform added; 0.01 *N* reagent obtained by tenfold dilution of stock solution and standardized against 0.01 *N* potassium iodate daily.

Potassium iodate, 0.1 *N*. 3.5569 grams of dried salt made to 1000 ml. with water; 0.01 *N* obtained by tenfold dilution.

Potassium chloride, 12.5 millimoles per liter. 0.9319 gram of dried salt made to 1000 ml. with 0.085 *M* phosphoric acid.

Sodium sulfide, 4% solution of the nonahydrate made in 250-ml. lots and kept refrigerated when not in use; stable for a few weeks.

Metaphosphoric acid, 20%. 20 grams of analytical reagent grade in 100 ml. of water prepared at room temperature and kept refrigerated.

Phosphate buffer, pH 7.4, 0.1 *M*, prepared according to Clark (*2*).

Hydrogen peroxide, 3%. Commercial solution kept refrigerated.

Petroleum ether, boiling point 30° to 60° C. Analytical reagent grade.

Sodium or potassium iodide, 10%. Prepared in 150-ml. quantities as needed and kept refrigerated; discarded when iodine can be detected by a test with starch indicator.

Silver iodate. Prepared as dry powder according to directions of Sendroy (*18*) and stored in amber glass-stoppered bottle in desiccator.

Starch indicator. Prepared according to Peters and Van Slyke (*11*).

Filter paper, chloride-free. 9-cm. Whatman No. 3 washed completely free from chloride with boiling water, dried, and stored in a closed container.

Frozen beef heart. A fresh beef heart is trimmed of fat, cut into three or four pieces, and stored in a deep freeze unit. The necessary quantity of tissue is obtained by shredding the frozen muscle on a household grater; the aconitase remains active for several months.

PROCEDURE

Preparation of Solution. Extracts suitable for the determination of citric acid in plant tissues are prepared as directed by Pucher, Wakeman, and Vickery (*16*). To suitable aliquots, 2 ml. of 18 *N* sulfuric acid are added and the solution, diluted to about 20 ml., is boiled for 5 minutes. After being cooled and filtered (most conveniently through a Gooch crucible into a beaker placed in a vacuum apparatus), 1 ml. of 20% metaphosphoric acid is added and the solution is diluted to 35 ml. and oxidized. Animal tissue extracts are deproteinized with 20% metaphosphoric acid according to the procedure of Krebs and Eggleston (*6*) and treated similarly, save that additional metaphosphoric acid is not needed.

Oxidation and Bromination. To the solution at a temperature not exceeding 22° C. are added 2 ml. of potassium bromide and 5 ml. of potassium permanganate; after about 10 minutes without stirring, the temperature is brought to about 10° C. by means of an ice bath, and the color is discharged by the rapid dropwise addition of ice-cold hydrogen peroxide with stirring. The solution is transferred to a 125-ml. pear-shaped separatory funnel and the beaker is rinsed with several small portions of petroleum ether totaling approximately 25 ml.

Great difficulty has been experienced in obtaining 125-ml. separatory funnels suitable for this operation. Funnels with outlet tubes of 8-mm. inside diameter and about 70 mm. long are required so that the aqueous phase will drain freely. It is usually essential to grind the stoppers to a perfect fit with the finest abrasive, if errors from the loss of petroleum ether are to be avoided. A trace of high grade grease is used for the stopcock but not for the stopper.

Isolation and Dehalogenation of Pentabromoacetone. The separatory funnel is shaken vigorously for at least 30 seconds. Stubborn emulsions occasionally formed with animal tissue extracts can be broken by the addition of a few drops of 2% aqueous solution of turkey red oil. The aqueous phase is drawn off quantitatively (a second extraction is unnecessary) and the ether is washed four times with 3-ml. portions of water to remove all halide ion. The pentabromoacetone is then decomposed by shaking the ether briefly with two successive 3-ml. portions of sodium sulfide, the colored solution being drained each time into a 25-ml. volumetric flask. The ether is washed with 2-ml. portions of water until no further color is removed, two washings being usually sufficient. The washings are drained into the same flask and 2 ml. of 2.0 *M* phosphoric acid are added. The solution is then boiled gently on a hot plate for 5 to 6 minutes. A few tiny chips of quartz previously extracted with acid and thoroughly washed are added just before heating the solution (not before adding the acid!) in order to facilitate smooth boiling. The flask is then cooled, *exactly* 5.00 ml. of 12.5 millimoles per liter of potassium chloride are added, and the solution is made to volume.

Titration of Halide. The contents of the flask are poured without rinsing into a 50-ml. conical flask which contains 0.25 gram of dry silver iodate previously measured into it with a glass spoon made for the purpose. The flask is stoppered and shaken on a platform shaking machine for 5 minutes and the suspension is poured through a dry chloride-free filter paper in a funnel. Suitable aliquots of the clear filtrate (usually 2 or 5 ml.) are at once pipetted into 100-ml. test tubes and 2 drops of 0.085 *M* phosphoric acid and 1 ml. of 10% sodium or potassium iodide are added. After approximately 5 minutes, the liberated iodine is titrated with 0.01 *N* thiosulfate by the customary technique, using starch as indicator. It is standard practice to titrate three aliquots.

Table II. Recovery of Bromide from Pentabromoacetone and of Citric Acid[a]

Citric Acid Taken *Mg.*	No. of Detns.	Bromide Found %	Citric Acid Found *Mg.*	Recovery %
3.213	4	98.30	3.242	100.32
2.154	9	98.13	2.157	100.51
2.106	7	98.01	2.104	99.91
2.093	6	98.03	2.094	100.05
2.056	2	97.83	2.052	99.81
1.077	2	97.48	1.071	99.44
1.053	5	97.93	1.052	99.91
1.047	3	97.88	1.045	99.86
0.5265	4	98.24	0.527	100.15
	42	Av. 98.036 ±0.315		99.99 ±0.31

[a] Average value 98.0% of theoretical yield of pentabromoacetone was used to compute citric acid found. Standard deviation is calculated for 42 determinations.

A blank determination on each lot of sodium sulfide solution must be run. Six milliliters of sodium sulfide, 5 ml. of water, 2 ml. of 2 *M* phosphoric acid, and a few tiny quartz chips in a 25-ml. volumetric flask are boiled on the hot plate to expel hydrogen sulfide and carried through the subsequent procedure as described. The blank titration obtained includes the value for the added potassium chloride as well as any halide in the reagent, and four or more individual titrations are made and averaged.

Calculation. The citric acid equivalent to the titration value is obtained from the following equations:

$$\frac{\text{Titration value} \times \text{normality factor} \times 1000}{6 \times \text{ml. of aliquot}} = \text{millimoles of iodate (or bromide) per liter} \quad (1)$$

$$\text{Millimoles found} - \text{millimoles in blank} = \text{net millimoles of iodate (or bromide) per liter} \quad (2)$$

$$\frac{\text{Net millimoles of iodate per liter}}{5 \times 40 \times 0.980} = \text{millimoles of citric acid per 25 ml.} \quad (3)$$

$$\frac{\text{Net millimoles of iodate per liter} \times 0.9606}{0.980} = \text{mg. of citric acid per 25 ml.} \quad (4)$$

The factor 0.980 represents the average percentage recovery of pentabromoacetone and is supported by the data in Table II. It rests upon 42 determinations which had a coefficient of variation of 0.32%. The factor 0.9606 is one fifth of the molecular weight of citric acid (as pentabromoacetone gives 5 equivalents of bromide) divided by 40, the ratio of 25 ml. to a liter. Ordinarily, citric acid will be computed in milligrams by Equation 4; however, Equation 3 is required to give the data used for the computation of isocitric acid described in a later paragraph. The final result is expressed in terms of the tissue by using the factor for the aliquot of the tissue extract originally taken.

DISCUSSION

Sendroy has shown that if the iodate ion concentration is less than 3 millimoles per liter, the application of a correction for the solubility of silver iodate is desirable. To avoid the necessity of employing solubility curves, the conditions in the present procedure have been adjusted so that the solution never contains less than 2.5 millimoles of iodate per liter. The preliminary experiments of Pucher showed that the solubility correction could be neglected under these conditions. Accordingly, 2.5 millimoles per liter of potassium chloride are added to the solution after the hydrogen sulfide is expelled. The titration of this chloride along with any halide in the reagents furnishes the blank subtracted from all determinations.

Table II shows a summary of a series of experiments on the recovery of citric acid over the range 0.5 to 3.2 mg. However, even smaller quantities can be determined if the potassium chloride is omitted and corrections for the solubility of silver iodate as estimated by Sendroy are included in the calculations. The mean recovery of bromide from the pentabromoacetone was 98.04% of theory with a standard deviation of ±0.315 or 0.32%. If this figure is employed in the calculation as shown in the equations, the mean recovery of citric acid is quantitative.

DETERMINATION OF ISOCITRIC ACID

Procedure. Extracts of plant tissues prepared as described by Pucher, Wakeman, and Vickery (*16*) contain an excess of alkali and acid is required in addition to the phosphate buffer of pH 7.4 to adjust the reaction correctly. A suitable aliquot of the extract is transferred to a 30-ml. beaker together with 5 ml. of pH 7.4 phosphate buffer solution. Roughly 1 gram of grated frozen heart muscle tissue is added and stirred into suspension with a rod, and the mixture is titrated to pH 7.4 with 0.05 *N* nitric acid using a glass electrode. The volume of nitric acid required is noted.

A similar aliquot of the extract is transferred to a 40-ml. graduated centrifuge tube, and 5 ml. of phosphate buffer and the amount of 0.05 *N* nitric acid determined as above are added; 1 gram of heart muscle tissue is stirred in and the volume is made to 15 ml. with water. After the suspension has been thoroughly mixed, the tube is stoppered with a plug of cotton and is incubated at 38° to 40° C. for 1 hour. To the warm suspension, 8 to 10 ml. of 20% metaphosphoric acid are added, and the solution is cooled to room temperature, adjusted to a volume of exactly 25 ml. with water, and centrifuged. A tissue blank is treated likewise, water being substituted for the extract, and 10-ml. aliquots are taken for oxidation.

The clear deproteinized solution is decanted and a suitable aliquot (10 or 5 ml.) is taken for the determination of citric acid by the procedure described, save that it is not necessary to add further metaphosphoric acid nor to boil and filter the solution after the addition of the 18 *N* sulfuric acid.

The tissue blank needs to be determined only occasionally; it is small and remains constant during the period that the heart is usable. The true blank arising from the muscle tissue is the difference between the tissue blank and the blank on the sodium sulfide solution. In practice, this difference may be employed in finding the tissue blank when a new solution of sodium sulfide is prepared.

Table III. Recovery of Bromide from Pentabromoacetone Obtained by Oxidation of Citric Acid in Presence of Bromide and Heart Muscle Extractives

Citric Acid Taken *Mg.*	No. of Detns.	Bromide Found %	Citric Acid Found *Mg.*	Recovery %
2.056	5	98.03	2.056	100.00
1.977	4	97.65	1.970	99.65
1.439	8	98.45	1.447	100.55
	17	Av. 98.14 ±0.43		100.06

That the recovery of pentabromoacetone is not affected by substances present in the extract of the heart muscle employed as the source of aconitase is evident from the data in Table III. These tests were carried out on the supernatant fluid obtained after incubating samples of heart muscle in the course of the determination of tissue blanks. No additional metaphosphoric acid was added. The recovery of pentabromoacetone was 98.14% of theory with a standard deviation for 17 experiments of ±0.43, a result indistinguishable from that in Table II. The recovery of citric acid was also quantitative.

Calculation. The net millimoles per liter of iodate (or bromide) are computed by deducting the tissue blank or, otherwise, the sum of the blank on the sodium sulfide and the true blank arising from the heart tissue, from the millimoles per liter found by titration. Due allowance is made if the tissue blank represents an aliquot different from that used for the determination. The number of millimoles of citric acid per 25 ml. is then obtained from Equation 3. As has been pointed out by Krebs and Eggleston, if this quantity is denoted by X and if Y is the quantity of citric acid in an identical aliquot of the extract before treatment with aconitase and, further, if Z is the combined quantity of *d*-isocitric and *cis*-aconitic acid present originally in the aliquot, one may set up the equation

$$Y + Z = X + \frac{0.11}{0.89} X \qquad (5)$$

or

$$Z = \frac{X}{0.89} - Y$$

This equation depends upon the equilibrium relationship among citric, *cis*-aconitic, and *d*-isocitric acid in the presence of aconitase. Table IV summarizes a number of observations of the proportion of citric acid present when citric, *cis*-aconitic, or *d*-isocitric acids were individually allowed to reach equilibrium in the presence of the enzyme. The average is 88.9 ± 0.55%. Thus the total acidity from these three acids, after equilibration, is made up of citric acid and a quantity of *d*-isocitric and *cis*-aconitic acid together equal to 11/89ths of the citric acid found.

Table IV. Proportion of Citric Acid Present after Equilibration of Citric, *cis*-Aconitic, and *d*-Isocitric Acids with Heart Muscle Aconitase

Substrate	No. of Detns.	Equilibrium Concentration %	Standard Deviation
Citric acid	32	89.27	±0.59
d-Isocitric acid	24	88.71	±0.42
cis-Aconitic acid	4	88.64	±0.32
	60	88.93	±0.55

If *cis*-aconitic acid is neglected in the calculation, the values of X and Y in Equation 5 may be expressed in milligrams—that is, the citric acid is calculated from Equation 4 rather than from Equation 3.

Determination of Citric Acid at Equilibrium in Presence of Aconitase. In order to obtain support for the results of Krebs and Eggleston (*3*, *5*) on the position of the equilibrium in the presence of aconitase, carefully purified specimens of each of the acids were prepared and samples were treated with heart muscle as described; citric acid was then determined. The results are summarized in Table IV.

The citric acid employed was prepared by dehydrating analytical reagent material. Titration indicated a purity of 100%. *d*-Isocitric acid was purified by recrystallization of the lactone (*13*) from chloroform. The lactone was prepared from the dimethyl ester isolated from Bryophyllum leaves and melted at 156° to 157° C. Hydrolysis of specimens of lactone on the steam bath in the presence of a small excess of 0.1 *N* sodium hydroxide for 15 minutes opened the lactone ring and back-titration indicated a purity of 100%. It was observed, however, that satisfactory standard solutions of isocitrate could not be obtained by alkaline hydrolysis of specimens of the dimethyl ester. Although titration data indicated high or complete purity, such solutions gave low yields of citric acid after incubation with aconitase. This anomaly has not been explained.

cis-Aconitic acid anhydride was prepared according to Anschütz and Bertram (*1*) and obtained in large crystals of melting point 82° C., several degrees higher than was recorded by Malachowski and Maslowski (*7*). Nevertheless, in spite of many recrystallizations from benzene, the anhydride still retained an impurity. A satisfactory specimen of the free acid was secured by crystallization of the anhydride from moist ethyl acetate and chloroform as described by Krebs and Eggleston.

Analysis of Mixtures of Citric and Isocitric Acid. To illustrate the accuracy of the methods, Table V shows the results of determinations made upon three mixtures of citric and isocitric acid. These were single determinations, but were calculated from the results of triplicate titration of aliquots.

Table V. Recovery of Citric and *d*-Isocitric Acid from Mixtures

Citric Acid Taken	Isocitric Acid Taken	Citric Acid Found		Isocitric Acid Found	
Mg.	*Mg.*	*Mg.*	%	*Mg.*	%
0.6455	1.013	0.646	100.1	1.01	99.7
0.4303	1.351	0.432	100.4	1.35	99.9
0.8606	0.6753	0.863	100.3	0.670	99.2

Analysis of Plant Tissues. The results of duplicate determinations of citric acid in 11 samples of tobacco leaf and 10 samples of Bryophyllum leaf were subjected to statistical analysis to obtain an estimate of the precision of the method. The citric acid content ranged from 6 to 111 me. per 100 grams of dry tissue. The standard deviation of a single determination was computed from the 21 differences between duplicates as 0.60 me. per 100 grams. Because of the wide range of the citric acid content of the samples, the expression of the error of a single determination as a percentage of the mean has no great significance; however, the value was ±1.2%.

A similar analysis of a group of 15 pairs of duplicate determinations of isocitric acid in samples of Bryophyllum leaf gave the standard deviation of a single determination as 2.8 me. per 100 grams of dry weight. These samples were relatively constant in isocitric acid content, the range being from 131 to 176 me. per 100 grams. The error of a single determination was ±1.7% of the mean.

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Volumetric Determination of Thorium by High Frequency Titrimetry

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This investigation was undertaken to illustrate how conventional analytical procedures may be simplified or shortened by use of high frequency titration. An indirect volumetric method for the determination of thorium using high frequency titrimetry is described. Oxalic acid and thorium nitrate standard solutions are used. The end point is sharp, stoichiometric, and reproducible to a few hundredths of a milliliter of 0.025 *M* thorium nitrate. Some substances such as titanium and zirconium, which interfere quantitatively in gravimetric methods, may be present in small amounts without causing appreciable error. Thorium analyses may be carried out in a shorter time than by conventional methods, especially in the presence of certain interferences.

EXCELLENT reviews have been written on methods of analysis for thorium (*4*, *7*). The conclusion of some of the writers (*7*) is that volumetric methods for the determination of thorium are either involved or inaccurate. As a result, the more accurate but time-consuming gravimetric procedures (*9*, *11*) are usually used. Another difficulty of these gravimetric procedures is their lack of specificity; thus, careful and complete separation from many interferences is required prior to determination.

A good volumetric procedure for the determination of thorium is therefore desirable. This is particularly true of a volumetric procedure which possesses greater specificity than the accepted gravimetric ones, because the rigor of time- and effort-consuming separations might be reduced.

Many stable substances react to form insoluble precipitates or undissociated complexes with thorium, but most of these substances cannot be used to determine thorium volumetrically because no good means exist to locate the end points for the reactions.

Recently, a high frequency instrument has been described which is adequate for carrying out most types of titrations with high precision (*1*). The titration vessel containing the solution to be titrated is placed in the field of a high frequency oscillator, and changes in frequency of the oscillator are measured as the titration proceeds. A plot of frequency against volume of standard solution gives a titration curve with a break in it at the end point, much like a conductometric titration curve. The advantage of this procedure for establishing the end point is that no chemical indicators, electrodes, or physical contact of any sort with the solution are required. With such a procedure, any substance which reacts quantitatively with a sought-for substance could conceivably be used to determine it.

This work describes the investigation of several reagents for the titration of thorium. The use of oxalate as a volumetric reagent is given in detail. A study of interferences shows that some elements which interfere quantitatively in the gravimetric determination of thorium are tolerable in small but appreciable amounts in the volumetric procedure using high frequency titration. Separation procedures to remove such interferences are in some cases shorter than the accepted gravimetric ones.

CONDITIONS FOR TITRATION

Search for Reactants. A survey of the literature (*7*) indicated that fluoride, iodate, ferrocyanide, and oxalate might be suitable reagents for the volumetric determination of thorium. In addition, tartrate and citrate were studied because these are known to complex heavy metal ions. Hydroxide was avoided because of its tendency to coprecipitate other ions.

The titration of fluoride with thorium nitrate has been used extensively (*6*, *10*, *12*) as has the reverse titration (*3*). The authors' work indicated that fluoride might be a suitable reagent for the high frequency titration. However, titrations of fluoride with standard thorium, using the conditions of Rowley and Churchill (*10*), gave titration curves with extreme curvature in the region of the equivalence point. It was impossible to locate the end point on precisely these curves. The curvature was not eliminated by varying conditions, such as acidity and thorium concentration, nor by titration of thorium with fluoride in the reverse manner.

This curvature is interpreted to mean that more than one thorium fluoride complex exists at the equivalence point corresponding to formation of thorium tetrafluoride. The end point is not sharp because the reaction between fluoride and thorium probably does not proceed according to a single equation. This interpretation is supported by observations of other workers. Dodgen and Rollefson (*3*) state that thorium and fluoride form several complex ions in dilute solutions. Clifford (*2*) found that the end point formed by using alizarin red as an indicator for the titration of fluoride with thorium is not sharp, but gradual. In any case, this reaction is definitely not useful for high frequency titrations.

The titration curves for thorium in acid solution with citrate, tartrate, citric acid, and tartaric acid exhibited no sharp end points and were badly curved.

Titration of thorium with iodate indicated that iodate might be a useful reactant. However, there was enough curvature in the region of the end point to interfere with precise location. This was probably due to the fact that thorium iodate is not very insoluble.

Ferrocyanide was entirely unsatisfactory, for it gave no breaks in the region of the equivalence point.

Sodium oxalate gave excellent titration curves with sharp end points which were reproducible under a variety of conditions. On this basis, a titration procedure for thorium involving standard oxalic acid was worked out.

Reagents. Reagent grade sodium oxalate was dried and used as a primary standard to prepare 0.05 *M* solution. This solution was checked within 0.1% against Acculute 0.1 *N* potassium permanganate (Sargent & Co.). Oxalic acid solution (0.05 *M*) was prepared from reagent grade oxalic acid and standardized against the standard permanganate.

Thorium nitrate solutions (0.025 *M*) were prepared by dissolving Baker's reagent grade thorium nitrate in water containing 0.2 to 5 ml. of 6 *M* nitric acid per liter to prevent hydrolysis. These solutions were standardized according to the usual gravimetric procedure (*11*), in which the thorium was precipitated as the oxalate and ignited to the oxide for weighing.

Direct Titration of Thorium with Standard Oxalate. Standard 0.025 *M* thorium nitrate (25-ml. portions) was titrated directly

with standard 0.05 *M* sodium oxalate in end-point volumes of about 125 ml. The end point was sharp and reproducible within a few tenths of a per cent (0.02 to 0.05 ml.), but the observed volume of thorium nitrate required was about 1% (0.25 to 0.30 ml.) below the theoretical.

This was in accord with the findings of Gooch and Kobayashi (*5*), who worked out a procedure for the volumetric determination of thorium in which the thorium sample and excess oxalic acid were mixed; the thorium oxalate was removed, and then standard permanganate was used to determine either the excess oxalic acid left in solution, or the oxalate on redissolution of the precipitate. These authors found that when the oxalic acid was added to the thorium, observed values of the thorium were low by about 1%. The reverse procedure of adding thorium to the oxalic acid gave results accurate within 0.1%.

Rider and Mellon (*8*) have also studied the oxalate precipitation process in connection with the indirect colorimetric determination of thorium. Excess standard oxalate was added to precipitate the thorium. The excess oxalate was determined indirectly after removal of the precipitate by adding excess standard permanganate and determining the excess permanganate colorimetrically. Rider and Mellon concluded that the order of addition of thorium and oxalate had no effect on the accuracy, providing there was always excess oxalate at the end of the precipitation process. These findings are not necessarily in contradiction to those described above, for the procedure of Rider and Mellon was empirical and the stoichiometry among thorium, oxalate, and permanganate was not investigated.

In the determination of thorium, the addition of the thorium sample to the standard oxalate was considered more cumbersome than the direct titration of the thorium sample with standard oxalate. For this reason, conditions were varied for the direct titration in an attempt to reduce or eliminate the 1% discrepancy between the theoretical and observed end points. The discrepancy could be varied but not satisfactorily eliminated, using the measures which follow.

First, the acidity at the end point was systematically reduced by partial neutralization of the excess acid in the thorium nitrate solution. As the pH at the end point increased, the observed end point still came too soon and deviated increasingly from the equivalence point. This was presumed a result of the precipitation of basic thorium salts.

The acidity at the end point could not be increased while using sodium oxalate as a titrating agent. When the pH fell below 3, the sharpness of the end point was reduced owing to combination of oxalate with the free acid which was present. With oxalic acid as a standard agent, this difficulty was eliminated. For pH values from 1.2 to 3.2 at the end point, the discrepancy remained constant at 1% when oxalic acid was used as the standard solution. This indicated that the discrepancy was not simply due to formation of basic salts.

The constancy of the discrepancy with widely varying acidity led to the hope that an end point correction might be applied, if the pH were regulated between 1.2 and 3.2. However, titration of different-sized samples of thorium showed variation of the discrepancy, but the variation did not seem to be proportional to the thorium content.

The authors believe that the discrepancy resulted from an irreversible coprecipitation of thorium on the thorium oxalate, possibly as thorium nitrate. In such a case, it seemed that introduction of various anions other than nitrate might have some effect on the magnitude of the discrepancy. Aliquots (100-ml.) of the standard thorium nitrate were converted to other salts such as thorium chloride, thorium perchlorate, and thorium sulfate by precipitating the hydroxide with ammonia, washing, dissolving the hydroxide in a 10% excess of the appropriate acid, and making the solution up to 100 ml. in a volumetric flask. Titration of 25-ml. portions of the resulting thorium solutions with standard oxalic acid still showed differences between the observed and theoretical end points. The discrepancy was bad in the sulfate solution, amounting to about 10%.

Using the thorium chloride solution, the conditions of titration did not have much effect on the discrepancy—for instance, boiling the solution after adding a major part of the standard oxalic acid in the cold, or adding the major part of the oxalic acid to the solution of thorium chloride at boiling temperature, and then completing the titration at room temperature, gave the same 1% discrepancy observed when the whole titration was carried out at room temperature.

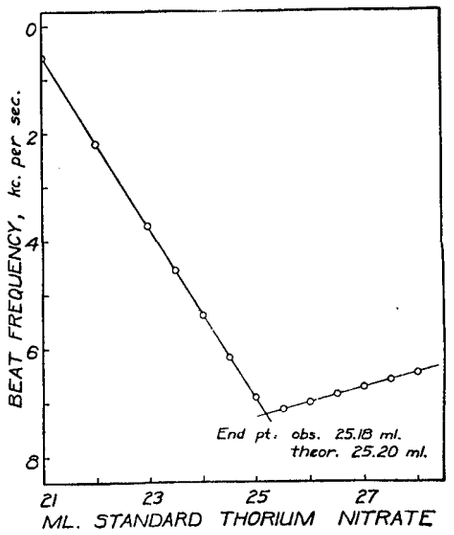

Figure 1. Titration of 25 Ml. of 0.04972 *M* Oxalic Acid with 0.02466 *M* Thorium Nitrate

The attempt to carry out this direct titration was abandoned after these experiments. The direct titration of thorium with standard oxalic acid could probably be performed with an error of the order of 0.5% by proper selection of conditions and simple application of corrections. For higher accuracy, the indirect method described in the next section must be used. The measures used to reduce the error were not exhausted. A more thorough study might result in an elimination of the discrepancy and lead to a simple, direct procedure for titrating thorium with standard oxalic acid.

Indirect Titration of Thorium Solutions. On the basis of the work of Gooch and Kobayashi (*5*), the high frequency method was used to follow the titration of aliquots of standard oxalic acid with standard thorium nitrate. The end points were sharp and agreed excellently with theoretical ones calculated from the standard concentrations, as shown in Table I. Conditions, such as speed of titration and presence of various inert salts, were not critical. Acidity was not critical for larger sample sizes (about 15 ml. or more of thorium nitrate). However, for smaller sample sizes (5 ml. of thorium nitrate) it was necessary to make the solution about 0.01 *M* in nitric acid. Titration at much lower or higher acidities resulted in extreme curvature and obliteration of the end point; however, this was no disadvantage in the indirect titration procedure described below. A typical titration curve is shown in Figure 1. The procedure adopted consisted of adding the thorium nitrate to an excess of standard oxalic acid. The excess oxalic acid was then determined by high frequency titration with standard thorium nitrate without removing the precipitate.

For any indirect titration, it is an advantage to know how much reagent to add in order that only a moderate excess be present. In the indirect titration of thorium, a simple and rapid method was found (*3*) for estimating the proper amount of standard oxalic acid to add to a particular sample. When oxalic acid

Table I. Titration of Oxalic Acid with Thorium Nitrate Solutions

(Aliquots of 0.04972 *M* oxalic acid were titrated at room temperature with 0.02466 *M* thorium nitrate containing 0.002 *M* nitric acid in an end-point volume of 125 ml.)

Determination	Vol. Thorium Nitrate Used, Ml.		
	Observed	Theoretical	Error
1	25.18	25.20	−0.02
2	25.57	25.55	+0.02
3	25.53	25.52	+0.01
4	25.25	25.26	−0.01
5	25.31	25.31	0.00
6	10.18	10.20	−0.02
7	10.10	10.08	+0.02
8	5.12	5.10	+0.02

Table II. Volume of 0.05 *M* Oxalic Acid Necessary to Produce Permanent Precipitate on Addition to 0.025 *M* Thorium Nitrate

(Various aliquots of thorium nitrate were diluted with water and titrated with oxalic acid. Volumes half way to the equivalence point were about 50 ml. in all cases.)

Determination	Vol. of 0.05 *M* Oxalic Acid, Ml.	
	Added to produce precipitate	Equivalent to 0.5 of thorium present
1	No ppt.	0.5
2	No ppt.	1.0
3	2.5	2.0
4	4	4.0
5	8	8.0
6	14	16.0

was added to thorium nitrate, the first perceptible and permanent precipitate formed when about one half the equivalent quantity of oxalate was added, within a milliliter or two, regardless of the size of the thorium sample above a certain minimum, as shown in Table II. An aliquot of the thorium nitrate sample was used in this way to determine roughly the equivalent amount of standard oxalic acid, and this aliquot was then rejected. A second aliquot of the thorium nitrate sample was then measured for exact titration, and added to 5 to 10 ml. more than the equivalent amount of oxalic acid in the titration vessel. The back titration was then carried out with standard 0.025 *M* thorium nitrate.

There are several advantages of this procedure over many accepted indirect ones:

1. A rapid test indicates the amount of oxalic acid required for a proper excess.
2. The thorium oxalate precipitate need not be filtered off before back-titration.
3. The procedure is strictly stoichiometric. This means that time-consuming gravimetric standardization of the thorium nitrate is not necessary, for oxalic acid may be used as a primary or secondary standard.

INTERFERENCES

The analysis of thorium ores is usually preceded by lengthy separation steps in which the thorium is precipitated and separated as several different compounds to remove interferences. Electrolysis in a mercury cathode cell removes many common interferences such as iron, bismuth, lead, silver, and mercury (*8*). The iodate process is among the best for removal of rare earths, but zirconium and titanium tend to come through with thorium (*7*). These, however, are removed in the oxalate precipitation. Scandium appears to come through both of these procedures (*7*). Since these substances interfere quantitatively in the gravimetric determination, they must be removed quantitatively.

To study the effect of a particular interference, a known quantity was added to a 20-ml. aliquot of 0.02461 or 0.02457 *M* thorium nitrate. The resulting sample was then analyzed by adding it to a 25-ml. aliquot of 0.04972 *M* oxalic acid and titrating the excess oxalic acid with standard 0.02457 *M* thorium nitrate. Both positive and negative ion interferences were studied; the results are shown in Table III. The table is self-explanatory, except for the case of scandium. Here, the only obtainable preparation was a scandium-yttrium mixture as the oxides, with an average atomic weight of 70 for the metals.

Any cation which complexes or precipitates oxalate may theoretically interfere, as may any anion which reacts with thorium. Also, coprecipitable anions or cations which form soluble thorium or oxalate salts may theoretically interfere. The modes of these interferences may be radically different.

Lanthanum, as representative of the rare earths, interferes quantitatively, as do scandium and yttrium. A single, sharp end point, similar to that in Figure 1, is obtained in titrations of

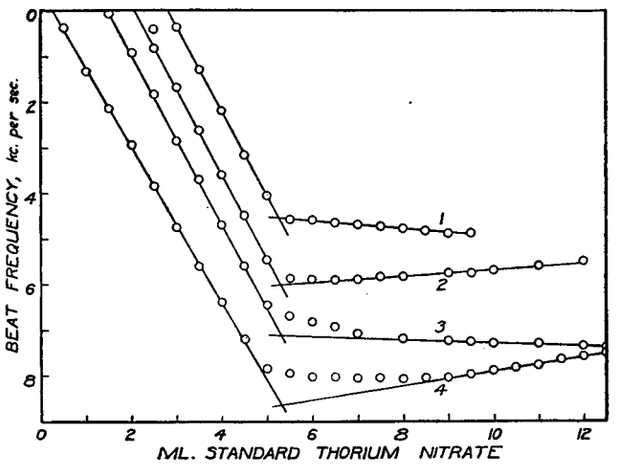

Figure 2. Effect of Zirconium on Volumetric Determination of Thorium

Conditions. 20-ml. portions of 0.02457 *M* thorium nitrate were mixed with small portions of 0.0025 *M* $ZrOCl_2$. These portions were then added to 25 ml. of 0.04972 *M* oxalic acid and back-titrated with 0.02457 *M* thorium nitrate

Curve	Mole Zr per Mole Th	End Points in Back-Titration, Ml.		
		Observed	Theoretical	Error
1	0	5.27	5.27	0.00
2	0.005	5.29	5.27	0.02
3	0.010	5.28	5.27	0.01
4	0.020	5.30	5.27	0.03

Table III. Interferences in Volumetric Determination of Thorium Nitrate

(Interferences were added to 20-ml. aliquots of 0.02461 *M* or 0.02457 *M* thorium nitrate. The samples thus prepared were added to 25-ml. aliquots of 0.04972 *M* oxalic acid and the excess oxalic acid was titrated with 0.02457 *M* thorium nitrate.)

Interference	Moles of Interference per Mole of Thorium	Moles of Thorium in Sample × 10^3		Error	
		Found	Theoretical	Mole	%
Zirconium oxychloride	0.005	0.4910	0.4914	−0.0004	−0.10
	0.010	0.4912	0.4914	−0.0002	−0.05
	0.015	0.4929	0.4914	+0.0015	+0.30
	0.020	0.4909	0.4914	−0.0005	−0.10
Titanium dioxide in H_2SO_4	0.025	0.4917	0.4922	−0.0005	−0.10
	0.050	0.4934	0.4922	+0.0012	+0.25
	0.100	0.492 ± 0.006	0.4922	±0.006	±1.2
Lanthanum sulfate	0.067	0.5155	0.4922	+0.0233	+4.6
Scandium and yttrium oxides in HCl	0.034	0.5045	0.4922	+0.0123	+2.5
Sulfate as H_2SO_4	0.5	0.4915	0.4922	−0.0007	−0.15
	1.0	0.4917	0.4922	−0.0005	−0.10
Acetate as HOAc	0.5	0.4917	0.4922	−0.0005	−0.10
	1.0	0.4910	0.4922	−0.0012	−0.25
	2.0	0.4915	0.4922	−0.0007	−0.15
Phosphate as phosphoric acid	0.10	0.4907	0.4922	−0.0015	−0.3
	0.20	0.4915	0.4922	−0.0007	−0.15
Tartrate as $NaKC_4H_4O_6$	0.20	0.491 ± 0.005	0.4922	±0.005	±1.0
Fluoride as KF	0.20	...	0.4922	...	....

mixtures of these substances and thorium. The end point apparently corresponds to the sum of these substances.

Zirconium interferes, but not quantitatively. It introduces a curvature into the titration curve, particularly in the region of the thorium equivalence point. When small, this curvature does not seem to introduce systematic error in location of the end point, but rather interferes with its precise location. In order to establish the end point in the presence of such curvature, it is necessary to draw the curve over a greater range than when curvature is absent (compare curves 1 and 4 in Figure 2). Considerable amounts of zirconium up to about 2 mole % of the thorium present are tolerable before the error in location of the thorium equivalence point exceeds a few tenths of a per cent. For larger amounts of zirconium, systematic error seems to appear. Titration curves are shown in Figure 2.

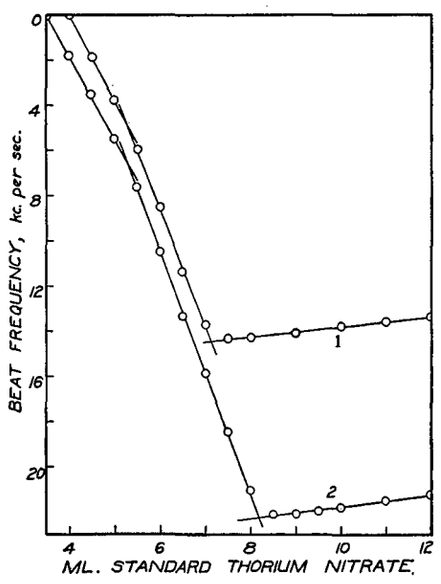

Figure 3. Effect of Phosphate on Volumetric Determination of Thorium

Conditions. Similar to those in Figure 2. Curves 1 and 2. 0.1 and 0.2 mole of phosphate per mole of Th, respectively; observed end points in back titrations, 5.33 and 5.30 ml., respectively. Theoretical end points, 5.27 ml.

The interference of titanium is similar to that of zirconium, but higher levels seem tolerable. Titanium up to about 5 mole % of the thorium present is tolerable before the error exceeds a few tenths of a per cent. As the amount of titanium increases above this, location of the end point not only becomes more uncertain because of curvature of the titration curve, but systematic error seems to appear. This behavior of zirconium and titanium is supported by the observations of Rider and Mellon (*8*).

Fluoride in fairly large amounts introduces a curvature into the titration curve that makes it impossible to locate the end point.

Sulfate is tolerable up to equimolar amounts compared to thorium, with an error less than a few tenths of a per cent. In quantities larger than this, results for thorium become too low, indicating coprecipitation. Acetate is similar to sulfate, but even larger amounts are tolerable. This property is not exhibited by chloride, nitrate, iodide, and perchlorate; large excesses of these are tolerable without interference.

The behavior of phosphate, which precipitated thorium in acid solution, was unforeseen. The titration curves in Figure 3 show breaks in the region corresponding to the thorium equivalence point. These breaks, while definite, are not great. In this sense, phosphate should be classed as an interference. The second breaks apparently correspond to reaction of the phosphate with

Table IV. Removal of Interferences in Volumetric Determination of Thorium

(Conditions for separations are as described in text, except that oxalate precipitations were omitted in determinations 1 to 4.)

Determination	Mole of Interference per Mole of Thorium			Mole of Thorium in Sample × 10^3			
						Error	
	La	Zr	Ti	Theoretical	Found	Mole	%
1	...	...	...	0.4890	0.4880	−0.0010	−0.2
2	...	...	...	0.4890	0.4885	−0.0005	−0.1
3	1.0	...	...	0.4890	0.4900	+0.0010	+0.2
4	1.0	...	...	0.4890	0.4885	−0.0005	−0.1
5	...	1.0	...	0.4890	0.4875	−0.0015	−0.3
6	0.5	...	0.4	0.4890	0.4875	−0.0015	−0.3
7	1.0	...	0.8	....	0.4880	−0.0010	−0.2

thorium, and seem quite sharp. There may be potentialities in the use of this reagent to titrate thorium in the presence of rare earths, for phosphates of the latter are soluble at acidities existing at the end points in Figure 3. This subject is being investigated further. Tartrate is similar to phosphate, except that neither of the two breaks is sharp.

This study of interferences is not intended to be complete; its purpose is to show how different interferences behave. The behavior of potential interferences cannot be safely predicted and must be examined experimentally before the volumetric method is used in their presence.

Removal of Interferences. Because appreciable quantities of many kinds of ions are tolerable, the oxalate titration in certain cases allows simplification of existing separation procedures (*9*). This is illustrated by the following analyses, which involve two oxalate precipitations and two iodate precipitations, followed by a hydroxide metathesis before titration. Analyses in the presence of various interferences are summarized in Table IV. The time required for duplicate determinations, including separation and titration, is only about 4 hours. The saving in time over accepted separation procedures (*9*) arises chiefly through the use of the centrifuge instead of filtration, and in the elimination of time-consuming measures required for complete separation or constancy of composition of the desired precipitates.

The determinations summarized in Table IV were performed as follows: The sample, containing about 0.5 millimole of thorium and an indeterminate amount of acid, was put into a 50-ml. centrifuge tube and neutralized with concentrated ammonia to the point of precipitation of hydroxides. The acidity was adjusted by adding 0.5 ml. of concentrated nitric acid followed by 3.5 grams of oxalic acid dihydrate. The use of one motor-driven stirrer (1-mm. diameter borosilicate glass rod) for each sample was a great aid in adding reagents and stirring up precipitates. The volume at this point was about 40 ml. The solution was brought to incipient boiling after addition of the oxalic acid, and the tube and contents were cooled to room temperature in an ice bath before centrifugation. Settling was not complete when the heating was omitted. After centrifugation at about 2700 r.p.m. for 2 minutes, the supernatant was removed and the precipitate was washed with 35 ml. of 2% oxalic acid containing 0.1% nitric acid. The wash liquid was removed by centrifugation. After solution of the oxalate precipitate in 10.0 ml. of concentrated nitric acid, 10 ml. of water and 3.5 grams of oxalic acid were added. The acidity was adjusted by adding an amount of concentrated ammonia equivalent to 9.5 ml. of the concentrated nitric acid. To prevent loss by spattering owing to boiling on neutralization, the tube was held immersed in a beaker of ice and the ammonia was added in small portions. After cooling to room temperature, the oxalate precipitate was centrifuged and washed.

The oxalate in the washed precipitate was not destroyed by a time-consuming calcination (*9*) before precipitating the iodate. Instead, the oxalates were dissolved in 20 ml. of concentrated nitric acid. Then 20 ml. of water and 3 grams of solid potassium iodate were added. (The amount of potassium iodate was not critical; it was added with a small measuring spoon. This was also true of the solid oxalic acid.) After cooling in an ice bath to room temperature, the mixture was centrifuged, the supernatant was removed, and the precipitate was washed with 35 ml. of iodate wash solution containing 8 grams of potassium iodate and 50 ml. of concentrated nitric acid per liter. The washed iodates were dissolved in 25 ml. of concentrated nitric acid and 20 ml. of

water, heated almost to boiling. The iodate was reprecipitated by adding 3 grams of potassium iodate, and was cooled, centrifuged, and washed.

The iodate was metathesized to the hydroxide by stirring with 35 ml. of 1 *M* ammonia, centrifugation, and removal of the supernatant. The bulk of the hydroxide was transferred to a measured excess of standard 0.05 *M* oxalic acid in the titration vessel. The centrifuge tube and stirring rod were washed with 3 to 4 drops of concentrated nitric acid and as much water as was necessary to effect quantitative transfer. The titration vessel was then inserted in the oscillator, a few minutes were allowed to reach equilibrium, and the titration was then completed with 0.025 *M* standard thorium nitrate. Metathesis of the hydroxide to the oxalate in the titration vessel appeared rapid and quantitative. In some cases the iodate was introduced to the titration vessel without metathesis to the hydroxide first, but the titration of the iodate was not always as satisfactory as that of the hydroxide.

ACKNOWLEDGMENT

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Cold-Surface Collection of Volatile Atmospheric Contaminants

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The analysis of contaminated atmospheres is often complicated by the low concentrations involved. However, condensation on cold surfaces can often be used to concentrate volatile materials. The sampling of volatile atmospheric contaminants by cold-trap techniques was studied in order to determine the efficiency of such methods and to develop improved equipment. Formaldehyde at concentrations in air of a fraction of a part per million was collected with about 80% efficiency at liquid nitrogen temperatures using metal packing in the traps. Without packing, only 50% efficiency was achieved. Similar results were obtained with low concentrations of benzene and sulfur dioxide. A convenient cold-trap system was developed consisting of a dry ice–cooled coil followed by a liquid nitrogen–cooled, metal-packed U-tube. The results of this work have been very helpful in studying the composition of smog in Los Angeles County and should be applicable to many air pollution problems.

DURING the course of an investigation of air pollution in the Los Angeles area it became evident that cold-trap techniques would be extremely valuable for the collection from the air of certain volatile contaminants such as aldehydes, ketones, and organic acids. The condensation of volatile contaminants by drawing them over cold surfaces is a well-known technique, but surprisingly little is known about the efficiency of such methods, particularly for the recovery of substances present in air at fractions of a part per million. Goldman and Dalla Valle (*1*) have discussed the efficiency of a dry ice–acetone trap for the collection of water vapor and bromine at relatively high concentrations.

The work described herein consisted of a study of the effect on the efficiency of cold-trap collection devices of changes in design, of the use of liquid nitrogen as well as dry ice as the cooling agent, and of the use of glass and metal packing in the traps. Efficiencies were determined for the collection of sulfur dioxide, formaldehyde, and benzene. The vapor pressure of sulfur dioxide at the boiling point of liquid nitrogen is about 3×10^{-13} mm. of mercury and that of benzene is about 1×10^{-18} mm. of mercury. These values were estimated by extrapolation from data presented by Lange (*2*) using the equation $\log_{10} P_{\text{mm. of Hg}} = \frac{-52.23\,A}{T} + B$. Thus, the collection efficiency of these substances at the concentration used in this investigation (greater than 0.1 p.p.m.) was limited by the apparatus rather than by the vapor pressure.

APPARATUS I

A cold-trap train which was used extensively in the Los Angeles area is shown diagrammatically in Figure 1. Figure 2 is a photograph of this apparatus mounted on the chamber in which the contaminated atmospheres were prepared.

The apparatus consisted of three 1-liter glass Dewar flasks mounted one above the other in a cascade system. Below the flasks was a more conventional trap consisting of an inlet tube extending nearly to the bottom of an outer tube. This lowest trap was about 35 cm. long and 3 cm. in diameter. The three flasks and the bottom trap were connected in series by means of ball-and-socket glass joints. Air was drawn between the walls of the flasks, through the bottom trap, through a wet-test gas meter, and finally through a vacuum pump. The Dewar flasks were filled with dry ice–isopropyl alcohol mixtures to cool the air passing between the walls. The outer surfaces of the Dewar flasks were insulated by placing them in boxes or cans and filling the remaining space with glass wool. The fourth and lowest trap was cooled by immersing it in a 1-liter Dewar flask containing either a dry ice–isopropyl alcohol mixture or liquid nitrogen.

Table I. Collection Efficiency of Various Modifications of Cascade-Type Cold-Trap System[a]

Operating Conditions	Formaldehyde, 20-Cubic Foot Samples: P.p.m. by wt. in air, detd. by bubbler sampling	P.p.m. by wt. in air, detd. by cold trap recovery	Recovery by cold traps, %	Benzene, 12-Cubic Foot Samples: Theor. p.p.m. in air	P.p.m. by wt. in air, detd. by cold traps	Recovery by cold traps, %
No packing	0.139, 0.106	0.073, 0.053	52, 50	89.0	53.3	60
Glass-bead packing in last trap	0.284	0.185	65	....	....	..
Stainless steel-washer packing in last trap	0.230, 0.246	0.196, 0.195	85, 79	89.0	80.8	91
Glass-bead packing in last trap, operated at 0.5 atm. pressure	0.172	0.092	53	....	....	..
Dry ice–isopropyl alcohol coolant used for all 4 traps	0.102	0.024	24	....	....	..

[a] Except where otherwise indicated, coolant in the 3 Dewar-type flasks was dry ice–isopropyl alcohol and the 4th trap was cooled by liquid nitrogen. Sampling rate in all cases was 20 cubic feet per hour.

EXPERIMENTAL

The collection efficiency of this cold-trap train for formaldehyde and benzene vapor was determined under various operating conditions.

Known concentrations of formaldehyde (0.1 to 0.3 p.p.m. by weight) in air were prepared in a chamber of 10 cubic meters in volume, through which air was moving at a rate of 5 cubic meters per minute. This chamber has been described in detail in other reports from this laboratory (*4*, *6*). Formaldehyde vapor, prepared by dripping a dilute solution from a motor-driven hypodermic syringe into a hot flask, was mixed with the air stream before it entered the chamber. As a check on the amount of formaldehyde in the chamber atmosphere, the air was drawn through two fritted-glass bubblers in series which contained a 1% sodium bisulfite solution. Previous work in this laboratory has shown this sampling method to be almost 100% efficient for the collection of formaldehyde. The formaldehyde recovered by both bubblers and freeze-out train was measured by the chromotropic acid method described by MacFadyen (*3*). In all experiments the sampling rate through the freeze-out train was approximately 20 cubic feet per hour, and sampling was continued for about 1 hour.

Benzene (about 90 p.p.m. by weight) was vaporized in the air stream entering the test chamber; the concentration of benzene in the chamber was estimated from the rate of vaporization and the rate of air flow through the chamber. The amount of benzene retained by the traps was determined by measuring the optical density at 254 mμ of an aliquot of the combined solution collected and the washings. The benzene was completely soluble in the water which was simultaneously collected. This relatively high concentration of benzene was used to provide a measurable absorption at 254 mμ.

The results are shown in Table I. Glass beads in the last trap increased the efficiency from 50 to about 65% and the use of metal packing (9-mm. diameter, stainless-steel washers) increased the recoveries to about 80%. It is probable that the low collection efficiency of the unpacked train was due partly to the formation of a fine mist that was not retained by the walls of the traps. The visible mist disappeared when either glass or metal packing was used.

In an attempt to increase the efficiency of the apparatus without the use of packing, an electrostatic precipitator was built into a trap, immersed in liquid nitrogen, and placed at the end of the train. This precipitator trap collected a considerable quantity of organic material, but this material had been chemically changed by the electrical discharge. (This was shown by the yellow-brown color which developed in place of the usual purple in the chromotropic acid–formaldehyde analysis; also, the ultraviolet absorption curve of the condensate from a benzene-contaminated atmosphere was quite different from the absorption curve of benzene, showing stronger absorption in the 230 to 250 mμ range.)

Several milliliters of liquid oxygen accumulated in the liquid nitrogen–cooled trap during the 1-hour sampling periods. During thawing, the escaping oxygen carried with it a part of the contaminants which were collected. The amount of formaldehyde lost in this manner was determined by passing the gases that escaped on thawing through a series of two fritted-glass bubblers containing a 1% sodium bisulfite solution, then analyzing this solution by the chromotropic acid method. With the unpacked trap system, 6% of the formaldehyde in the air sampled was lost from the traps during this gas blowoff in each of the two trials. When steel washers were used as packing, 0.5% was lost in each of two tests.

Oxygen condensation was prevented during one run by maintaining the interior of the liquid nitrogen trap at 0.5 atmosphere pressure with the aid of stopcocks on each side of the trap. The formaldehyde recovery dropped to 53%, making such an arrangement undesirable.

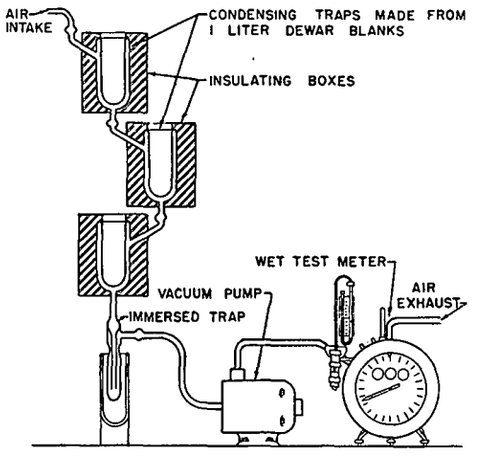

Figure 1. Schematic Drawing of Cascade Cold-Trap Device

One test was made in which the dry ice–isopropyl alcohol mixture was used throughout (replacing liquid nitrogen in the last trap). Only 24% of the formaldehyde was retained by the traps, 20% in the first three traps, and 4% in the last. Accordingly, in the experiments which follow, liquid nitrogen was used as coolant for the fourth trap; the use of dry ice–isopropyl alcohol was continued in the three Dewar-type traps.

APPARATUS II

The preceding apparatus was bulky and therefore difficult to use for field sampling. Consequently, a freeze-out system of quite different design was built (Figure 3).

This system consisted essentially of two parts; the first was a dry ice–cooled trap, the main function of which was to remove water from the air, and the second was a liquid nitrogen–cooled trap. The dry ice trap consisted of an 8-foot coil of 18-mm. outside diameter borosilicate glass tubing which was immersed in a dry ice–isopropyl alcohol mixture in a 1-gallon, wide-mouthed Dewar flask. This coil afforded approximately the same area of cooled surface for condensation as did the three Dewar traps of

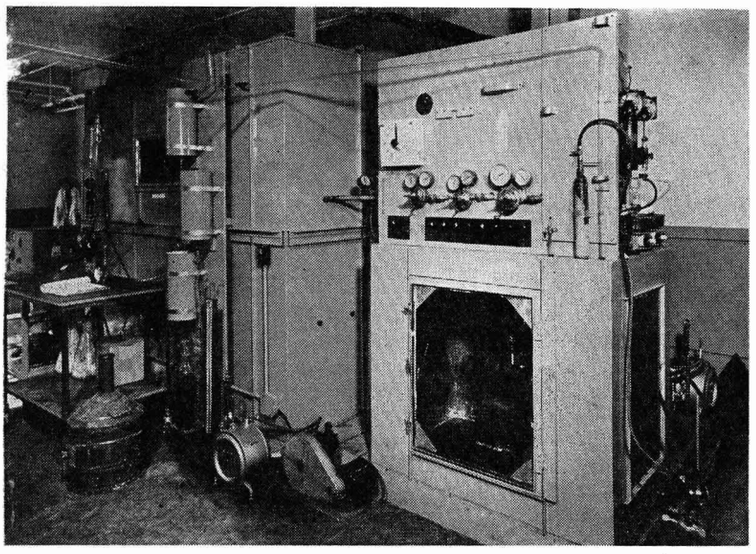

Figure 2. Cascade Cold-Trap Device

Mounted on side of chamber in which contaminants dispersed

Apparatus I. The liquid nitrogen trap consisted of a U-tube immersed in liquid nitrogen in a quart Dewar flask. The two arms of the U-tube were of greatly different size; the arm on the inlet side was 35-mm. outside-diameter glass tubing, and the outlet arm was 8-mm. outside-diameter tubing; the over-all length was 30 cm. The top of the large arm was connected to the spiral of the preceding trap by a ground-glass joint. The wide arm of the liquid nitrogen trap was filled with 9-mm. diameter stainless-steel washers. The use of a U-tube greatly simplified both the introduction of the packing material and the washing of it at the end of a sampling period. Air samples drawn through this system passed next through a gas meter and then through a vacuum pump.

EXPERIMENTAL

The efficiency of this cold-trap train was determined by using it to sample atmospheres, prepared as previously described, containing known amounts of formaldehyde and benzene vapors. The collection efficiency for sulfur dioxide was also found by metering this gas into the air stream entering the test chamber, and then sampling the resulting atmosphere. As with formaldehyde, the sulfur dioxide concentration in the chamber was checked by parallel sampling through two fritted-glass bubblers in series. In the case of sulfur dioxide, the bubblers contained a 0.1 *N* sodium hydroxide solution. In both the cold trap condensate and washings, and the bubbler solutions, measurement of sulfur dioxide content was made by oxidizing the sulfite to sulfate with hydrogen peroxide and analyzing the sulfate turbidimetrically after the addition of barium chloride, as in the method described by Sheen *et al.* (*5*). In all cases the sampling rate was 20 cubic feet per hour. After each sample was taken and the condensate was allowed to thaw and was removed from the traps, both the spiral trap and the packing in the liquid nitrogen trap were washed with three aliquots of distilled water, and the washings were added to the condensate for analysis. A total of about 150 ml. of condensate and washings was obtained by this procedure.

The results of these tests are given in Table II.

Because of the probability that additional contaminant could be collected by using additional traps in the train, a second washer-filled, liquid nitrogen–cooled U-tube was placed after the first, and the resulting series of three traps was used to collect formaldehyde. The condensate and washings from each trap were analyzed separately, and the recoveries were compared with recoveries obtained by bubbler sampling. The results are shown in Table III.

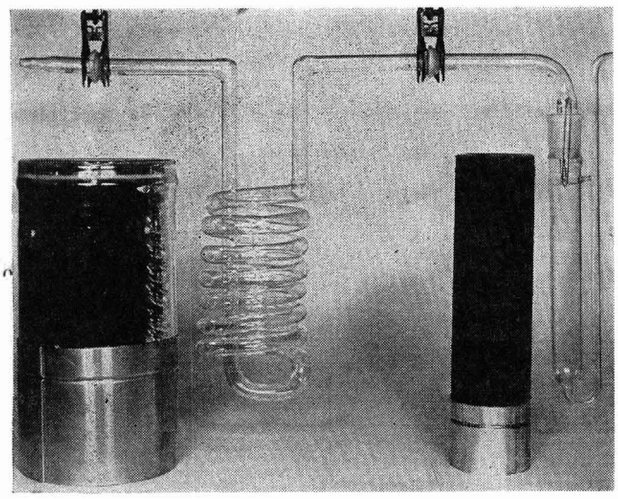

Figure 3. Simplified Cold-Trap Device

Table II. Efficiency of Second Type of Cold-Trap System for Formaldehyde, Benzene, and Sulfur Dioxide Collection

Contaminant	Sample Size, Cubic Feet	Concentration of Contaminant, P.p.m. by Wt.	Recovery by Cold Traps, %
Formaldehyde	13	0.362, 0.362	79, 77
Sulfur dioxide	12	2.8	64
Benzene	6	186	86
	13	169	93

Table III. Efficieney of Individual Traps in Second Type of System

Traps	Percentage of Total Formaldehyde	Percentage of Total Collected Formaldehyde
Dry ice	8	10
First liquid nitrogen	57	72
Second liquid nitrogen	14	18

DISCUSSION OF RESULTS

The results of this investigation indicate that fairly efficient collection of many volatile contaminants from contaminated atmospheres can be achieved by the use of a simple cold-surface condensation system. The presence of proper packing, such as the steel washers, considerably increased the efficiency. Probably the most important function of this packing was to increase the surface on which the volatile material could condense, thus decreasing the amount of condensation on nuclei in the air. This would prevent the formation of fogs which could escape from the traps.

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Determination of Lithium in Rocks

Fluorometric Method

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The gravimetric method in general use for the determination of lithium is tedious, and the final weighed product often contains other alkali metals. A fluorometric method was developed to shorten the time required for the analysis and to assure that the final determination is for lithium alone. This procedure is based on the complex formed between lithium and 8-hydroxyquinoline. The fluorescence is developed in a slightly alkaline solution of 95% alcohol and measurement is made on a photoelectric fluorometer. Separation from the ore is carried out by the wet method or by the distillation procedure. Sodium and potassium are removed by alcohol and ether, but complete separation is not necessary. Comparison of analyzed samples shows excellent agreement with spectrographic and gravimetric methods. The fluorometric method is more rapid than the gravimetric and produces more conclusive results. Another useful application is in the preparation of standard lithium solutions from reagent quality salts when a known standard is available. In this case no separations are necessary.

THE difficulties inherent in the gravimetric determination of lithium in minerals have been reviewed by Kallmann (*5*) and by Fletcher (*2*). In nearly all methods previously reported for this determination, partial separations from the other alkalies are made and a correction factor is used to compensate for the sodium and potassium remaining with the lithium; or very careful separations are made and the final residue is weighed as a pure lithium salt. However, even after a supposedly "complete separation," as in the sulfate gravimetric method, the final lithium sulfate residue contains traces of calcium and magnesium as well as some sodium and potassium. Needless to say, it would be far better to be able to determine the lithium directly instead of having to depend on the complete separation of all other ions.

In an effort to develop a direct method for the determination of lithium, a group of compounds was studied with the hope of finding a specific colorimetric or fluorometric reagent. Inasmuch as lithium has been frequently described as an interference in reactions involving beryllium, attention was directed to those compounds (chiefly the hydroxyanthraquinones) that had been reported (*8*) to give either direct color or fluorescence responses with beryllium. None of these compounds was found sensitive enough to serve for quantitative purposes; but oxine (8-hydroxyquinoline) formed an intensely fluorescing chelate complex with lithium and appeared to be the best reagent available.

Oxine will give a quantitative response with as little as 5 micrograms of lithium oxide in 25 ml. of solution and does not form a fluorescent substance with either sodium or potassium. Lithium may be determined with this reagent to an accuracy of 1 part in 1000 in solutions that contain no other cations. Appreciable quantities of sodium decrease the intensity of the fluorescence of the lithium-oxine complex; therefore, a partial separation of the sodium is necessary. The advantages of using the oxine reaction for the determination of lithium are that a direct quantitative determination may be made without a complete separation of the other alkalies or the use of correction factors; and at the same time, greater accuracy is achieved in shorter time than is possible with the sulfate gravimetric determination.

REAGENTS AND APPARATUS

Alcohol. Redistilled 95% ethyl alcohol was used for the preparation of all solutions in the fluorometric procedure. Commercial alcohol contains a highly fluorescent substance, but redistillation from an all-glass still gives a nonfluorescent reagent that should be stored in glass-stoppered bottles. When 95% alcohol is mentioned in this paper, it refers to this redistilled product.

Absolute redistilled alcohol was used for separations of sodium and potassium.

Standard Lithium Solution. LITHIUM CARBONATE. Reagent-grade lithium carbonate was recrystallized twice from hot water according to the directions of Caley and Elving (*1*). This was analyzed by the sulfate method, and the value obtained was used as the lithium content. Spectrographic analysis showed that this sample contained less than 0.01% sodium oxide.

STOCK SOLUTION. A quantity of the purified lithium carbonate equivalent to 250 mg. of lithium oxide was dissolved in a small amount of dilute hydrochloric acid. This solution was evaporated to dryness, and one drop of concentrated hydrochloric acid was added. The lithium chloride was dissolved in 95% alcohol and made up to 250 ml. with alcohol. One milliliter of this solution was equivalent to 1 mg. of lithium oxide.

WORKING SOLUTION. The stock solution was diluted 1 to 10 with 95% alcohol after adjustment of the alkalinity. A predetermined amount of 0.01 *N* potassium hydroxide in alcohol was added to 10 ml. of the stock solution and the mixture was diluted to 100 ml. with 95% alcohol. One milliliter of this solution was equivalent to 1 microgram of lithium oxide. The solution may be diluted further if desired.

As indicators cannot be added to the solutions that are used for fluorometric work, the amount of potassium hydroxide required for adjustment of the alkalinity was determined by titration of a separate aliquot. For this, 10 ml. of the stock solution were mixed with 10 ml. of water and titrated to the methyl orange end point with 0.01 *N* potassium hydroxide in alcohol. The amount of potassium hydroxide used in this titration was the "predetermined amount" of potassium hydroxide added to the working solution. In many cases, the lithium chloride solution will show the correct acidity with methyl orange and it will not be necessary to add base.

Ether, absolute diethyl ether.

Reagent. A composition solution, 50 ml. of Solution A added to 25 ml. of Solution B, must be mixed fresh each day.

SOLUTION A. Oxine (8-hydroxyquinoline), 85 mg. dissolved in 250 ml. of 95% alcohol.

SOLUTION B. Alkaline acetate solution, 0.315 gram of potassium hydroxide and 0.20 gram of anhydrous potassium acetate dissolved in 250 ml. of water.

Potassium hydroxide, 0.01 *N* in alcohol, 0.28 gram of potassium hydroxide dissolved in 500 ml. of 95% alcohol.

Methyl Orange Indicator.

Sensitive Fluorometer. The fluorometer used in this investigation has been described (*4*). Corning filter No. 3486 was used with the blue-sensitive phototube of the Beckman spectrophotometer; however, Corning filters No. 3387 and No. 3780 also were satisfactory as secondary filters.

PROCEDURE

A 1- to 3-gram sample was decomposed, and the mixed alkalies were isolated from the other constituents by either the Fletcher (*2*) volatilization procedure or the Kallmann (*5*) method. In

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either case, calcium was removed as the oxalate, and after excess oxalate was destroyed with aqua regia, any zinc or traces of magnesium were separated with oxine. For this step, the mixed alkali chlorides or perchlorates were dissolved in a small amount of water, and 2 ml. of a 5% oxine solution in acetic acid were added; this solution was made alkaline with ammonium hydroxide, heated, and allowed to stand. At the end of 2 hours, the solution was filtered and the precipitate was washed with dilute ammonium hydroxide. The filtrate was then evaporated to dryness with aqua regia, followed by nitric and perchloric acids to destroy organic matter. If the Fletcher (*2*) method is used, magnesium is not distilled; therefore if zinc is absent the oxine separation is unnecessary.

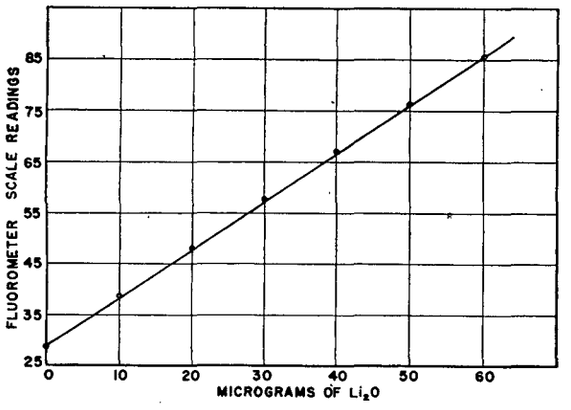

Figure 1. Standard Curve for Fluorescence of Lithium with 8-Hydroxyquinoline

After the isolation of the alkalies, the lithium was separated from the bulk of the other alkalies by the Palkin alcohol-ether method, as described by Scott (*7*). The mixed salts were dissolved in a minimum of water (less than 2 ml. if possible), and 1 drop of concentrated hydrochloric acid was added. Twenty milliliters of absolute alcohol and 60 ml. of absolute ether were added with swirling, and the mixture was allowed to stand for 5 minutes with occasional mixing. The solution was then filtered by suction and the precipitate was washed well with a 1 to 5 mixture of alcohol and ether. The lithium-bearing filtrate was evaporated to dryness on a steam bath. When the sodium and potassium precipitate was extremely large—i.e., when more than 2 ml. of water was required to dissolve the mixed alkalies—a second separation was made. The lithium residue was dissolved in 10 ml. of absolute alcohol, and 50 ml. of ether were added with swirling. One drop of concentrated hydrochloric acid was then added, and the mixture was set aside and swirled occasionally. At the end of 0.5 hour the solution was filtered with suction and the filtrate was evaporated to dryness. One drop of concentrated hydrochloric acid was added to the lithium chloride residue, which was then dissolved in 95% alcohol and made up to 100 ml. in a glass-stoppered volumetric flask. This solution was used for the fluorometric determination after adjustment of its alkalinity and proper dilution.

FLUOROMETRIC DETERMINATION

The alkalinity of the alcoholic lithium solution was adjusted to duplicate that of the standard. Again, indicators could not be added to the solutions used for the fluorometric measurements, so a 5-ml. aliquot of the sample solution was diluted to 15 ml. with water and titrated to the methyl orange end point with 0.01 *N* potassium hydroxide in alcohol. Five times this amount of potassium hydroxide was added to a 25-ml. aliquot of the sample solution and the mixture was made up to 50 ml. with 95% alcohol in a volumetric flask.

A sample solution that contained about 10 micrograms of lithium oxide per milliliter was found convenient for the fluorometric determination. Hence, the approximate concentration range of the sample was evaluated to determine whether or not further dilution was necessary. To accomplish this, 3 ml. of the oxine reagent were added to 0.1 ml. of the adjusted sample solution in a 25-ml. glass-stoppered volumetric flask (or glass-stoppered graduated cylinders) and the mixture was made up to volume with 95% alcohol. The solution was thoroughly mixed and the fluorescence measured. The final sample solution was then prepared by further dilution of the adjusted solution if it proved necessary. When the dilution required was 1 to 5 or more, the magnesium ion did not constitute an interference, and the fluorometric measurements could be made directly on the diluted solutions after addition of the reagent. However, when a dilution of less than 1 to 5 was made, sodium fluoride was added to suppress the interference of magnesium as indicated below.

For the final quantitative determination, 0.5, 1.0, 1.5, 2.0, and 2.5 ml. of the diluted sample solution were transferred to 25-ml. volumetric flasks; 3 ml. of oxine reagent was added to each, and the mixtures were diluted to 25 ml. with 95% alcohol. For samples requiring sodium fluoride, 1 ml. of 95% alcohol saturated with sodium fluoride was added to each flask. The fluorescence was measured, and the readings were interpolated from a standard curve. This curve was prepared from similar quantities of the standard solution and was made anew for each batch of reagent mixture. A typical working curve is shown in Figure 1.

NOTES ON THE PROCEDURE

Pipets should be used for measuring volumes. If burets are desired, a silicone lubricant should be used on the stopcock, as ordinary stopcock grease is fluorescent.

Each batch of distilled alcohol should be tested for fluorescence with a visual comparator of the type previously described (*3*).

EXPERIMENTAL RESULTS

Four rock samples, analyzed for lithium at four different laboratories, were analyzed by the fluorometric method at the Bureau of Mines. These results are compared in Table I.

Table I. Determination of Lithium Oxide in Analyzed Samples

Sample	Lithium Oxide, %				
	Analyses by Four Laboratories				Fluorometric
Lithium ore	4.89	..	..	..	4.95[a]
Lithium ore	1.37	1.78	1.48	1.80	1.46[b]
Lithium ore	1.10	0.92	1.30	1.11	1.04[b]
Lithium ore	1.10	0.92	1.30	1.11	1.06[a]
Lithium ore	0.58	0.65	0.87	1.63	0.68[a]
Lithium ore	0.13	0.15	0.32	0.38	0.17[a]

[a] Separation using distillation method of Fletcher (*2*).
[b] Separation using method of Kallmann (*5*).

The first value for each rock was obtained by spectrographic analysis and the other three by gravimetric procedures. These data show that the fluorometric method produces results similar to those of the spectrographic method and within the range of the standard procedures. As a further check, synthetic samples were prepared to contain known amounts of lithium, and were analyzed by the fluorometric method. Typical results are indicated in Table II. Synthetic samples 1 and 2 were duplicates and contained the equivalent of 0.175 gram potassium oxide, 0.144 gram sodium oxide, 0.03 gram magnesia, 0.05 gram of calcium oxide, 0.01 gram of ferric oxide, 0.01 gram of alumina, 0.001 gram of lead oxide, 0.001 gram of zinc, 0.001 gram of manganese oxide, and 0.2065 gram of lithium oxide. Sample 3 contained the above, but only 0.025 gram of lithium oxide. Sample 4 contained lithium oxide and magnesium oxide only. Aliquots of these were analyzed for the lithium oxide content.

Table II. Determination of Lithium Oxide

Sample	Li_2O in Aliquot, γ	Li_2O Found, γ	Deviation, γ
1	57.7	56.7	1.0
2	33.0	32.0	1.0
3	37.5	39.0	1.5
4	22.0	23.0	1.0

CHARACTERISTICS OF FLUORESCENCE

When exposed to ultraviolet light, mixtures of lithium and oxine produce a greenish fluorescence in weakly alkaline alcoholic solutions. The wave length of the band of emitted light covers the range of 4900 to 5700 A. and the intensity of the fluorescence

is a function of the concentration of the lithium. The fluorescence develops to a maximum almost instantly and remains stable for several days in stoppered flasks.

The intensity of the fluorescence varies with changes in hydrogen-ion concentration, and, as these are alkaline solutions, large amounts of carbon dioxide or acid fumes in the laboratory will affect the fluorescence if the solutions are exposed to air for long periods. Alkalinity is controlled by adjustment of the neutrality of the sample and by the addition of definite quantities of potassium hydroxide and potassium acetate in the reagent. The effect of alkali on the fluorescence is indicated in Figure 2. The fluorescence increases sharply with an increase in alkalinity and then decreases to a rather constant value. This region of stability was selected as the best alkalinity at which to work, even though it did not represent the point of maximum fluorescence.

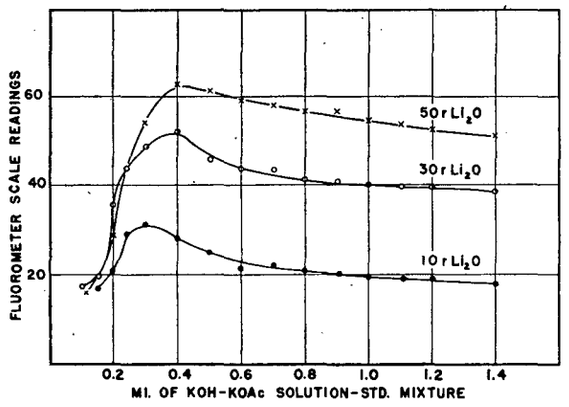

Figure 2. Effect of Alkali on Lithium-8-Hydroxyquinoline Fluorescence

The presence of even very small amounts of water caused a marked decrease in the intensity of the fluorescence; therefore, the water content must be controlled carefully. If the aqueous content of the alcohol is disregarded, water is added only in the reagent mixture. All glassware must be dry and rinsed with alcohol before use.

The oxine concentration likewise affects the intensity of the fluorescence. This is illustrated in Figure 3. Although there is some latitude in the oxine concentration that will give consistent results, 2.0 ml. of the 0.034% oxine solution were chosen as the optimum quantity for the range of lithium determined.

The fluorescence intensity is stable to ordinary laboratory light and to normal laboratory temperature changes.

EFFECT OF OTHER IONS

Sodium ions in concentrations up to 1.0 mg. of sodium chloride in 25 ml. caused no fluorescence with the oxine reagent.

As much as 1.0 mg. of sodium chloride caused little change in the readings on 10 or 30 micrograms of lithium oxide. Over 0.1 mg. caused a decrease in the 50-microgram sample. This is illustrated in Figure 4.

As the amount of sodium chloride left in the solution after the separation described is far less than this amount, it causes no difficulty. In the early stages of development of this procedure, sodium hydroxide was used in place of potassium hydroxide in the reagent, and the results were equally as good as when potassium hydroxide was used.

Potassium ions up to a concentration of 1.0 mg. of potassium chloride in 25 ml. had no effect upon the fluorescence of lithium solutions. This is illustrated in Figure 5.

Magnesium. Oxine is a very sensitive fluorometric reagent for magnesium, and as little as 0.3 microgram of magnesium

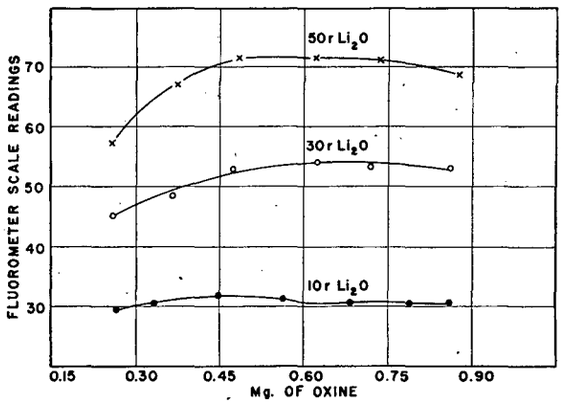

Figure 3. Effect of 8-Hydroxyquinoline Concentration

oxide in 25 ml. will cause a positive error in a lithium determination. Experiments showed that an ammonium hydroxide–ammonium carbonate separation left intolerable amounts of magnesium oxide in solution. The oxine separation often leaves as much as 100 micrograms of magnesium oxide in solution; but with the dilution technique recommended, this amount of magnesium oxide will not interfere if the solution obtained after adjustment of neutrality is diluted at least 1 to 5. This technique overcomes the magnesium interference whenever the original sample contains 10 mg. or more of lithium oxide.

Low-grade samples that require a final dilution of less than 1 to 5 are likely to contain amounts of magnesium oxide greater than 0.3 microgram in the aliquot used for the fluorometric determination of lithium oxide. However, addition reagents may be used for these samples to overcome the magnesium interference.

Several addition reagents such as the fluoride, oxalate, borate, and hexametaphosphate ions were tried to test their efficiency in suppressing the magnesium fluorescence. Oxalate had no effect upon the fluorescence of either magnesium or lithium; borate caused a slight decrease in the fluorescence of magnesium but had no effect upon lithium; the hexametaphosphate caused a decrease in the fluorescence of both magnesium and lithium; sodium fluoride greatly suppressed the fluorescence of magnesium and had very little effect upon that of lithium, provided some magnesium was present. The fluoride, therefore, appeared to be the best suppressant. Typical results obtained through the use of fluoride ions are presented in Table III. In the first four figures of Table III it will be noted that sodium fluoride did not repress the lower aliquots sufficiently but repressed the upper ones too much. The readings of four or five aliquots are necessary for a good average.

The addition of alkali reduces the fluorescence of the magnesium-oxine complex much more than that of lithium. Therefore, when this method is used to determine microgram quantities

Table III. Effect of Fluoride Ion on Fluorescence of Lithium-Oxine Complex in Presence of Magnesium

Li_2O in Aliquot, γ	MgO in Aliquot, γ	Apparent Li_2O Found without Addition of NaF, γ	Li_2O Found after Addition of NaF, γ
10	1	25	12
20	1	35	21
30	1	45	29
40	1	53	37
44	[a]	50.5	43.7
10	[a]	11.1	9.7

[a] MgO remaining in solution after oxine separation of magnesium and zinc.

of magnesium, the pH of the solutions should be much closer to the neutral point than is required for lithium determinations. The intensity of the fluorescence of the magnesium-oxine complex decreases rapidly on standing, in contrast to that of the lithium oxine complex, which is very stable. The measurement of the fluorescence of the magnesium-oxide complex was used to determine the amounts of magnesium left in solution after an oxine separation. The results so obtained checked very closely those obtained spectrographically on the same sample. The fluorometric method found 1 microgram of magnesium oxide per ml., as compared to 0.9 microgram per ml. found with the spectrograph. If the distillation method of separation is used, magnesium is effectively eliminated.

Calcium. Calcium oxide in amounts as large as 30 micrograms in 25 ml. had little or no effect upon the fluorescence of lithium solutions. Greater quantities caused a steady increase in the intensity of the fluorescence, but the increases were not sufficiently great to allow for a method for the accurate determination of calcium. The quantity of calcium left in solution after the oxalate separation amounted to about 0.3 mg. of calcium oxide. With the dilution technique, therefore, calcium does not constitute an interference. In fact, as much as one tenth of the original sample could be used before calcium would cause any appreciable error in the determination of lithium.

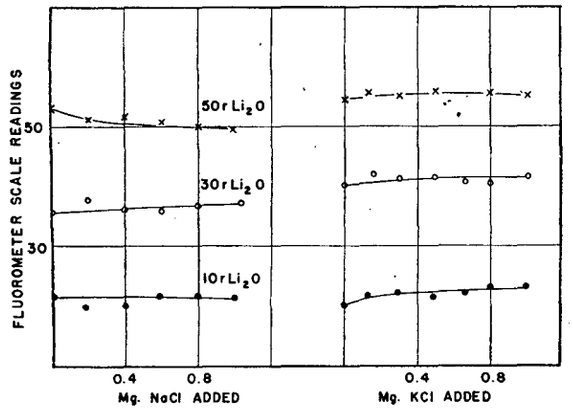

Figure 4. Effect of Sodium Chloride

Figure 5. Effect of Potassium Chloride

Zinc. Merritt (*6*) has shown that oxine is a very sensitive quantitative reagent for zinc. It is necessary, therefore, that the zinc be removed as far as possible.

Experiments showed that on starting with 0.046 gram of zinc, about 400 micrograms remained in solution after a hydrogen sulfide precipitation; after precipitation of 0.046 gram of zinc with oxine only 73 micrograms remained in solution. Tests also showed that if residues from these separations were made up to 100 ml. with alcohol, aliquots of the solution from the hydrogen sulfide separation greater than 0.2 ml. would disturb the lithium determination; more than 1 ml. of the solution from the oxine separation could be tolerated. In an actual determination not over 0.3 ml. of the sample solution would be used; therefore, oxine provides a safe separation. The amount of zinc left in solution by the hydrogen sulfide separation is much greater than the solubility product would indicate. This may be due to colloidal zinc sulfide passing through the filter; however, usual care was used in this procedure.

SEPARATION OF LITHIUM

Several solvents were investigated for the separation of lithium chloride from the chlorides of sodium and potassium. After extraction with the solvent, it was necessary to heat the mixture to remove the hydrochloric acid. Both amyl alcohol and *n*-butyl alcohol formed highly fluorescent substances on heating in the presence of the lithium chloride. If the solution was taken nearly to dryness, a tar was formed, and at 70° the *n*-butyl alcohol turned brown. It was found that by reducing the pressure and keeping the temperature below 50° C., the hydrochloric acid could be removed without formation of these objectionable materials. However, the process was too time-consuming for practical analysis. Ninety-five per cent ethyl alcohol as a separation solvent did not produce consistent results. The ether-alcohol separation, as outlined by Scott (*7*), gave excellent results. In this method, it was found that if less than 2 ml. of water were required to dissolve the initial precipitate, a second precipitation was not necessary.

COMPOUNDS TESTED AS REAGENTS

The compounds tested for color or fluorescence reactions with lithium were: 1,4-, 1,8-, and 1,5-dihydroxyanthraquinones, 1,2-5,8-tetrahydroxyanthraquinone, 1-amino-4-hydroxyanthraquinone, *p*-nitroazoresorcinol, 2-naphthylamine, cochineal, 1-amino-2-naphthal-4-sulfonic acid, *p*-nitrobenzeneazoresorcinol, morin, and quercetin. None of these proved sensitive enough for either qualitative or quantitative reagents for lithium.

ACKNOWLEDGMENT

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The authors wish to express their appreciation to M. J. Peterson for the spectrographic analysis and to H. W. Jaffe for the spectroscopic check of residues for lithium.

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RECEIVED May 18, 1950.

Determination of Nitrate in Plant Material—Correction

In the paper "Determination of Nitrate in Plant Material" by C. M. Johnson and Albert Ulrich [ANAL. CHEM., **22**, 1526–9 (1950)], the following errors in statistical terminology appear:

In Table I and also in Table II, footnote [b] should read, σ = standard deviation = $\sqrt{\frac{\Sigma(x - \bar{x})^2}{n - 1}}$, instead of σ = standard deviation = $\sqrt{\frac{x - \bar{x}}{n - 1}}$. The remainder of the footnote is correct.

In Table III, footnote [d], a portion should read, C.L. = mean $\pm t \frac{\sigma}{\sqrt{n}}$, rather than C.L. = mean $\pm t\sigma\sqrt{n}$. The remainder of the footnote is correct. All calculations were based on the correct formulas and are correct.

C. M. JOHNSON

Determination of Sodium and Potassium in Lithium Metal by Flame Photometer

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The unusual properties of lithium metal have caused the recent interest in its use in metallurgy. Sodium and potassium are the chief impurities usually found in lithium metal. In the course of work on the refining of lithium a rapid and accurate method was required for determining very small amounts of these contaminants. The flame photometer proved satisfactory for the analyses. It was possible to determine as little as 0.001% of both sodium and potassium. No separations were necessary, because the small negative error due to the presence of lithium could be dealt with by means of a correction curve. The analyses could be easily completed within 2 hours. The production of lithium of high purity for use in light alloys is of importance in metallurgy. In certain light alloys containing lithium the tolerance for sodium is very low and lithium is considered to be the major source of this element. The method could be modified for the determination of sodium and potassium in such alloys.

DURING the course of an investigation into the refining of lithium metal, at the Mines Branch (*8*), it was required to determine sodium in the range of 1% to as low as 0.001% and potassium to less than 0.01%.

Bills *et al.* (*3*) discuss the errors inherent in the classical gravimetric separation of sodium and potassium, which tend to give high false values for sodium when this element is present in very small amounts. Lindsay, Braithwaite, and D'Amico (*5*), using alcoholic magnesium uranyl acetate, found it possible to estimate sodium oxide in the order of 0.01% in the presence of up to twice the concentration of potassium or lithium salts. In the present investigation, however, lithium would be present in up to 100,000 times the concentration of sodium. Caley, Brown, and Price (*4*) showed serious interference by lithium and potassium in the determination of sodium by the triple acetate procedure, lithium interfering slightly more than potassium.

For the past two years, a Perkin-Elmer Model 52A flame photometer has been employed in these laboratories for alkali determinations, and the possibility of extending the use of this instrument to cover the above-mentioned ranges was considered. The manufacturer's literature (*7*) shows an interference by 1000 p.p.m. of lithium of 1 p.p.m. for potassium and 3 p.p.m. for sodium, in the propane flame. In the case of sodium it is not specified whether this interference is for the red-sensitive or the blue-sensitive photocell. In the authors' experience, commercial lithium salts and commercial lithium metal both may contain appreciable quantities of sodium and potassium, and might account for this positive error.

It has been found by Berry, Chappell, and Barnes (*1*), and confirmed by Parks, Johnson, and Lykken (*6*) and Bills *et al.* (*2*), that the presence of large concentrations of foreign salts produces negative errors with filter-type flame photometers (Perkin-Elmer Model 18). Working at low ranges, however, Bills *et al.* (*2*) experienced less interference, and found that by the use of correction curves compensating factors could be applied.

The purpose of this paper is to demonstrate that a rapid and reasonably accurate determination of small amounts of sodium and potassium in lithium metal is possible with the flame photometer; and to show the errors introduced by the relatively large amount of lithium sulfate which is present in the solution.

APPARATUS

The Perkin-Elmer Model 52A flame photometer was fitted with the propane burner, and Pyrofax gas was used as fuel. Potassium was measured with the red-sensitive (No. 918) phototube and sodium with the blue-sensitive (No. 1P37). Constant voltage was maintained by means of a Raytheon voltage regulator in the circuit between the line supply and the instrument.

An hour's warm-up, including 15 minutes with the burner lit, was allowed before making determinations, in order to attain maximum stability in operation.

REAGENTS

Sulfuric acid, reagent grade.

Methyl red, 0.1% alcoholic solution.

Stock Solution. Reagent grade sodium chloride and potassium chloride were dried at 110° C. for several hours. Then 2.5418 grams of the sodium chloride were dissolved in distilled water and made up to 1 liter, giving a concentration of 1000 p.p.m. in sodium. A 1000 p.p.m. potassium solution is obtained by weighing out 1.9069 grams of potassium chloride, dissolving, and bringing to a volume of 1 liter.

Standard solutions were made from the stock solutions by appropriate dilution with distilled water.

PROCEDURE

Calibration. As the standard curves were not straight lines, with the departure from linearity more marked at higher concentrations, it was necessary to plot sufficient values to obtain accurate working curves. The points taken for the ranges used in this work were as follows, for both the sodium and the potassium:

0 to 2 p.p.m. 0.10, 0.20, 0.50, 1.40, 1.70, 2.00
0 to 10 p.p.m. 2.0, 4.0, 5.0, 6.0, 8.0, 10.0
0 to 50 p.p.m. 5.0, 10.0, 15.0, 20.0, 25.0, 30.0, 35.0, 40.0, 45.0, 50.0

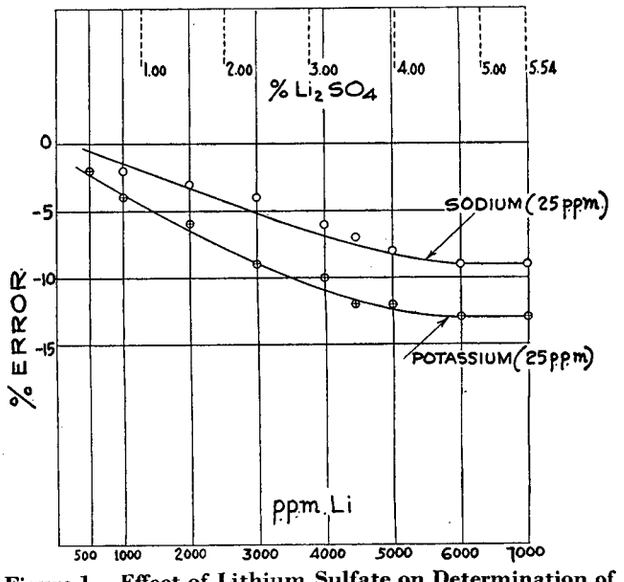

Figure 1. Effect of Lithium Sulfate on Determination of Sodium and Potassium

Table I. Sodium and Potassium in Lithium Metal

Sample	Sample Weight	Sodium: Photometer range	Sodium: Na detd.	Sodium: Blank	Sodium: Net Na	Sodium: Na	Potassium: Photometer range	Potassium: K detd.	Potassium: Blank	Potassium: Net K	Potassium: K
	Mg.	*P.p.m.*	*Mg.*	*Mg.*	*Mg.*	%	*P.p.m.*	*Mg.*	*Mg.*	*Mg.*	%
III, crude lithium	2278.1	0–50	12.2	0.02	12.2	0.54	0–2	0.12	Nil	0.12	0.005
	3078.2	0–50	16.5	0.02	16.5	0.54	0–2	0.16	Nil	0.16	0.005
50–9, partially refined lithium	2791.3	0–10	3.73	0.02	3.71	0.13	0–2	0.13	Nil	0.13	0.005
	1831.1	0–10	2.35	0.02	2.33	0.13	0–2	0.09	Nil	0.09	0.005
50–10, refined lithium	2077.4	0–2	0.040	0.02	0.020	0.001	0–2	0.10	Nil	0.10	0.005
	2623.7	0–2	0.045	0.02	0.025	0.001	0–2	0.13	Nil	0.13	0.005
50–8, refined lithium	3652.1	0–2	0.11	0.02	0.09	0.003	0–2	0.20	Nil	0.20	0.005
IV, refined lithium[a]	1677.6	0–2	0.07	0.02	0.05	0.003	0–2	0.12	Nil	0.12	0.007
V, refined lithium[a]	2106.1	0–2	0.06	0.02	0.04	0.002	0–2	0.12	Nil	0.12	0.006

[a] Commercial product guaranteed less than 0.005% Na.

Table II. Effect of Lithium on Determination of Sodium and Potassium

Li	Range	Sodium: ^{a}Na (by flame photometer)	Sodium: Na added	Sodium: Na recovered	Sodium: Error	Potassim: K present (by flame photometer)	Potassim: K added	Potassim: K recovered	Potassim: Error
P.p.m.	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*	*P.p.m.*
6943	0–2	0.18	...	...	...	...	0.35	...	...
6911	0–2	0.18	0.125	0.30	0	0.35	0.125	0.49	+0.01
6873	0–2	0.18	0.25	0.45	+0.02	0.35	0.25	0.63	+0.03
6804	0–2	0.18	0.50	0.73	+0.05	0.35	0.50	0.90	+0.05
3161	0–2	0.08	...	...	...	0.20	...	...	...
3131	0–2	0.08	0.50	0.61	+0.03	0.20	0.50	0.73	+0.03
3102	0–2	0.08	1.00	1.10	+0.02	0.20	1.00	1.23	+0.03
4777	0–2	0.17	...	...	...	0.24	...	...	...
4729	0–10	0.17	2.0	1.95	−0.22	0.24	2.0	2.05	−0.19
4681	0–10	0.17	4.0	3.85	−0.32	0.24	4.0	3.85	−0.39
4633	0–10	0.17	6.0	5.70	−0.47	0.24	6.0	5.60	−0.64
4538	0–10	0.17	10.0	9.20	−0.97	0.24	10.0	9.24	−1.00
4212	0–2	0.12	...	...	...	0.23	...	...	...
4170	0–50	0.12	10.0	9.5	−0.6	0.23	10.0	9.1	−1.1
4107	0–50	0.12	25.0	23.6	−1.5	0.23	25.0	22.6	−2.6
4036	0–50	0.12	40.0	37.2	−2.9	0.23	40.0	36.0	−4.2

While it was possible to have 2 p.p.m. read 100 on the meter, for both elements practically full gain was required. Therefore, in the 0 to 2 p.p.m. range, 80 was taken as the upper limit in order to allow for the small adjustments which are necessary during the course of several analyses. For the 0 to 10 and 0 to 50 ranges, the 100 reading was taken as the top of the curve. The zero point in all cases was set against distilled water.

Sampling. The lithium metal was sampled by the procedure of Rogers and Viens (*8*). Portions weighing from 1.5 to 4.0 grams were cut with a sharp steel chisel and placed in tared weighing bottles, all under an atmosphere of argon. The samples were allowed to come to room temperature, and were weighed. The lithium was transferred to the surface of ice (made from distilled water) in stainless steel beakers. The reaction was smooth and quiet. The resulting solutions were neutralized to methyl red with sulfuric acid, transferred to 500-ml. borosilicate glass volumetric flasks, and brought to volume with distilled water. Precautions to avoid contamination, as outlined by Bills *et al.* (*2*), were observed. Blanks containing the same amounts of ice, methyl red, sulfuric acid, and distilled water were prepared at the time of sampling.

Analytical Procedure. Determine the lowest range in which the unknown may be analyzed. Then set the zero point with distilled water, and the top of the curve with the standard solution, after which take a reading of the unknown; this gives a close approximation of the true value. To obtain the final figure for the sample, take the average of several readings, each time checking the calibration points immediately below and above on the reference curve.

EFFECT OF LITHIUM SULFATE

Known amounts of sodium and potassium were added to analyzed samples and the recoveries were determined in the appropriate ranges. The results are given in Table II. To measure the effect of varying the lithium concentration, 25 p.p.m. each of sodium and potassium were assayed in the presence of from 100 to 7000 p.p.m. of lithium, the higher value being equivalent to 5.54% lithium sulfate. These findings are plotted in Figure 1.

RESULTS AND DISCUSSION

Table I gives some typical analyses of crude and refined lithium. Good precision is indicated by the agreement of duplicates. The values for the blanks were constant.

From the data in Table II it will be seen that sodium and potassium, in the order of 0.01%, can be determined in lithium metal with an accuracy of within 10%, the error in all cases being on the positive side. As the limit of detection by the flame photometer for both sodium and potassium is about 0.02 p.p.m., the relative error will increase with diminishing amounts of the element being determined.

In the 0 to 2 p.p.m. range complete recoveries of added elements were achieved, while in the higher ranges suppression, amounting to about 10% for potassium and 7% for sodium, was experienced. Figure 1 demonstrates that this negative error increases with increasing concentration of interferent, although in this work there was a tendency for the percentage error to level off at about 6000 p.p.m. of lithium.

The greater accuracy in the 0 to 2 p.p.m. range is in agreement with the recommendation of Bills *et al.* (*2*), ".... for minimum interference from foreign solutes, flame photometers should be operated at the lowest possible range."

The suppression in the higher ranges generally confirms the findings of others (*1*, *2*, *6*). In this study, however, the amount of interference was less. This may be due to differences between the Perkin-Elmer Model 18, used in earlier work, and the 52A model as employed in this laboratory. The 52A is fitted with a monochromator. Furthermore, sodium is preferably determined with a blue-sensitive (1P37) photocell, which is more sensitive to the sodium flame than the 918 and is also practically insensitive to the propane lithium flame.

In consideration of the data in Table II, where essentially the same percentage error was encountered from 2 to 40 p.p.m., it is suggested that correction curves such as those shown in Figure 1 may be constructed and adjustments made to the values as read from the photometer. This procedure should yield results accurate to 2% of the amount present.

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Estimation of Nicotinic Acid in Tissues by the Cyanogen Bromide Reaction

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A number of procedures for estimating nicotinic acid by the use of cyanogen bromide were found to suffer from interfering colors, instability, and insensitiveness. A study of the factors influencing the cyanogen bromide procedures developed various conditions that improved the estimation. The reagent is best prepared from crystalline cyanogen bromide. The temperature of the reaction must not go above 40° C. for 5 minutes or 25° C. for 30 minutes of reaction time. The pH of the medium is very critical, as color development falls off very rapidly on either side of pH 4.9 to 5.3. The reaction is best conducted in the dark. When *p*-methylaminophenol is used as the amine, the chromogen produced is very stable over 4 hours. The procedure described, if carefully followed, is more selective and precise than other cyanogen bromide methods which have been applied to animal tissues for estimation of nicotinic acid.

IN PREPARING for a study of the effect of individual amino acid deficiencies, intake of all other dietary constituents being maintained at normal levels by forced feeding, chemical methods of estimation of nicotinic acid were sought which could be applied to the analysis of tissues. It was found that the published methods of colorimetric analysis suffered from interfering colors, instability, or insensitiveness. An improved modification of the cyanogen bromide method has now been developed, which largely overcomes these difficulties.

EXPERIMENTAL

Bandier and Hald's modification of the cyanogen bromide reaction (*1*, *2*) was used as a basis for this study, primarily because they claim greater stability of the chromogen produced with *p*-methylaminophenol sulfate than when other amines are used, and also because the color development is completed in an aqueous medium. However, it was hard to obtain consistent results. Using a Coleman Universal spectrophotometer, concentration curves were prepared by the original method. The galvanometer readings obtained within any one set of determinations checked fairly well, but the values obtained in different sets using the same nicotinic acid concentrations did not check. In other words, the K values obtained were not constant. The normal greenish-yellow color which should have developed was often masked by a reddish-brown color. Because the procedure did not give duplicable results on known nicotinic acid solutions, a study of the factors involved in this reaction was undertaken.

Cyanogen Bromide Reaction. EFFECT OF PURITY OF CYANOGEN BROMIDE. The cyanogen bromide reagent as generally used is made by adding cold 10% sodium cyanide to a cold saturated bromine solution until the solution is just colorless. The authors found that this procedure does not give a reproducible reagent. Apparently the eye is not sensitive enough to detect when all the bromine has been combined. Some "colorless" reagents developed an amethyst color on addition of the amine. This was responsible for off-color in the final reading and was thought to be due to a trace of free bromine which oxidizes the amine to a colored derivative. Adding a slight excess of sodium cyanide when preparing the reagent is claimed to overcome this defect (*3*). However, cyanogen bromide reagents prepared this way must be of slightly different compositions and therefore more difficult to buffer to the same pH. Roggen's procedure (*10*) of making the reagent was also tried, but constant readings could not be obtained. In agreement with Bandier and Hald (*2*), the preferred reagent was found to be a solution of crystalline cyanogen bromide, Eastman's No. 919, in water.

EFFECT OF TEMPERATURE. The effect of temperature on the cyanogen bromide reaction was found to be important (Figure 1). Most methods (*2*, *5*, *6*) allow this reaction to take place at a temperature of 75° to 80° C. The color which quickly developed at 75° C. decreased rapidly thereafter, and only when the temperature was not allowed to go above 40° C. was the greatest color obtained. Continuing the study, it was found that the maximum color developed if the reactants were allowed to react at 25° C. for 30 minutes.

A great part of the inconsistency obtained by the published

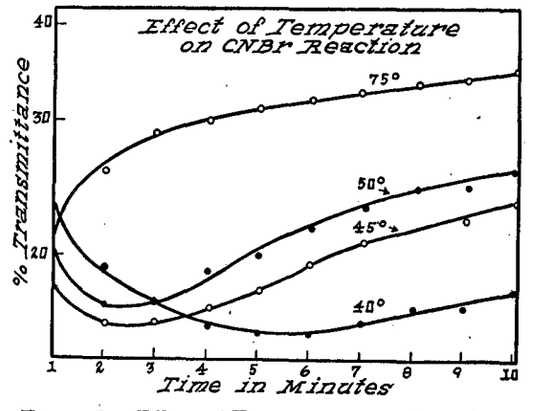

Figure 1. Effect of Temperature on Reaction

In each case from a series of tubes one was removed every minute, the color developed, and per cent transmittance determined

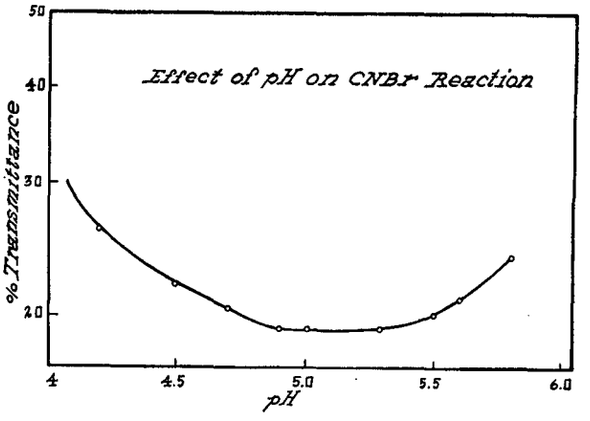

Figure 2. Effect of pH on Reaction

Duplicate sets of tubes were prepared over the pH range; one tube at each pH was used for pH measurement and the other for color development. Transmittance of one tube was plotted against pH of the other

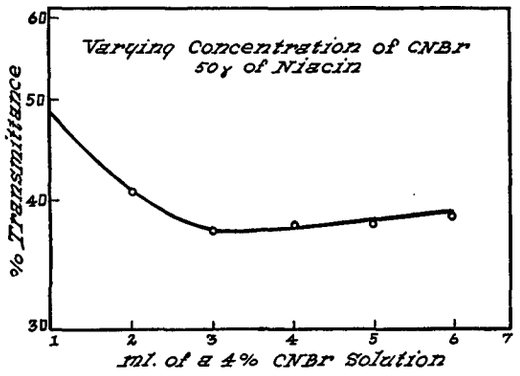

Figure 3. Effect of Cyanogen Bromide Concentration

Color developed according to procedure that did not give intensity finally achieved by method described in text

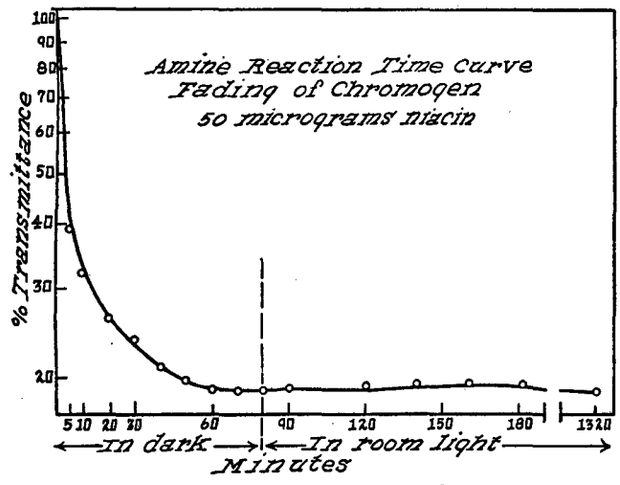

Figure 4. Fading of Chromogen

3 ml. of buffer, 4 ml. of cyanogen bromide reagent, and 14 ml. of *p*-methylaminophenol sulfate in 0.1 *N* sulfuric acid. Chromogen developed in darkness for 80 minutes and then left in light

methods appears to be due to the use of high temperatures, which cause loss of color. The tubes are not always in the water bath for the same time, and as this reaction is very sensitive to temperature, different values for the same nicotinic acid concentration are obtained. This can be avoided by using a temperature of 25° C. and allowing longer time for the reaction.

Effect of pH. The pH of the medium in which the cyanogen bromide reacts is very critical. In various procedures this reaction is carried out at pH from 4.3 to 7.5 (*6, 8, 9*). In these studies it was found that the pH range must be kept between 4.9 and 5.3 for maximum color development. On either side of these values color development rapidly declines (Figure 2). Because the reaction of the *p*-aminophenol sulfate with the cyanogen bromide–nicotinic acid complex requires a low pH, it was found impossible to combine the two reactions as recommended by Melnick and Field (*9*) in the case of aniline.

To control pH at this point phosphate–citric acid buffers were used (*7*): one of pH 5.25 for solutions containing protein-split products and one of pH 4.9 for pure aqueous solutions. The buffer must be so chosen that the pH is about 5.1 when the buffered solution of the sample and the cyanogen bromide reagent are added together. The control of pH is important for sensitivity and intensity of color development.

Effect of Cyanogen Bromide Concentration. The sensitivity of the reaction is also dependent to some extent on the concentration of cyanogen bromide used in the reaction. This concentration varies from 0.4 to 2.4% in different procedures (*2, 9*). The best color development was found when 4 ml. of a 4% cyanogen bromide reagent were made to a total volume of 10 ml. with the buffered solution of the unknown. This corresponds to a 1.6% concentration (Figure 3).

Effect of Light. When the reaction of cyanogen bromide was allowed to proceed in indirect daylight, the color was only 94% of that produced in the dark.

Reaction with Aromatic Amine. The chromogens produced by different amines vary in their stability to light. Martinek *et al.* (*8*) state that the color produced using aniline reaches a maximum in 5 minutes and then rapidly fades, whereas orthoform (methyl-*m*-amino-*p*-oxybenzoate) also reaches a maximum development of color in 5 minutes but is stable for about 15 minutes.

Harris and Raymond (*5*) and Kodicek (*6*) used *p*-aminoacetophenone as the amine. Their spectrophotometric readings had to be made within 15 minutes and their chromogens were not exposed to light.

The authors substantiated Bandier's claim that the chromogen produced using *p*-methylaminophenol sulfate is stable to light (*1, 2*). They left the tubes out in diffuse daylight for 24 hours with only very slight fading (Figure 4). The development of full color is relatively slow with this amine, taking about 80 minutes to reach full value. There was a slight increase in color intensity when the reaction proceeded in the dark. Allowing both the cyanogen bromide and amine reactions to proceed in the dark increased the color intensity 5.2%. There was also a slight increase in color intensity when the amine reaction proceeded at 15° C. Above pH 2 an off-color developed, but this was prevented when the amine reacted in a solution of pH 1.0. The amine reagent was therefore dissolved in 0.1 *N* sulfuric acid.

The *p*-methylaminophenol sulfate obtained on the market sometimes has a slight pink color in solution or develops one on standing 2 to 3 hours at room temperature. The impurity responsible was removed from the amine by washing with alcohol.

Preparation of Tissue Extracts for Analysis. In view of the effect of the factors mentioned above, a procedure has been developed for analysis of biological tissues and fluids.

Pepsin was used as a means of hydrolysis in preference to Bandier and Hald's (*1, 2*) alkali treatment or Dann and Handler's (*3*) acid treatment. This was done because the pepsin hydrolyzate could be used for the determination of other vitamins. Highly colored digests were avoided, so that color interference in the hydrolyzate was at a minimum.

A volatile solvent such as acetone was not used, for extraction of the nicotinic acid from the hydrolyzate, as large errors could result from evaporation of acetone.

One to 5 grams of minced tissue were placed in a 250-ml. Erlenmeyer flask. To this were added 0.5 gram of pepsin and 50 ml. of 0.1 *N* hydrochloric acid and the mixture was allowed to digest at a temperature of 37° C. in an incubator for 24 hours. This acid-pepsin digest was adjusted with 5 ml. of 1 *N* sodium hydroxide to a pH of approximately 5.2, quantitatively transferred to a 100-ml. glass-stoppered volumetric flask, made up to volume, and well mixed. Ten-milliliter aliquots were used for the determinations.

Of the reagents investigated for precipitation of the larger protein-split products, only zinc sulfate was satisfactory, as clear centrifugates were obtained (*4*) and no loss of nicotinic acid resulted from its use. The zinc sulfate is added to the hydrolyzate after alkaline hydrolysis of the nicotinic acid derivatives and subsequent partial neutralization.

METHOD

Reagents. Hydrochloric acid, 0.1 *N* and 1.0 *N*.

Sodium hydroxide, 1.0 *N*.

Zinc sulfate, 10%.

McIlvaine's buffer was prepared by making 15.2 grams of disodium hydrogen phosphate and 9.75 grams of citric acid ($H_3C_6H_5O_7.H_2O$) to 1000 ml. with water, checking on pH meter, and adjusting to the correct pH of 5.25.

Cyanogen bromide, 4.0%. It is best to use crystalline cyanogen

Table I. Nicotinic Acid Recoveries from Egg Albumin Digests

Nicotinic Acid Added	Nicotinic Acid Found	Nicotinic Acid in Aliquot Found		Recovery
γ	γ	γ	γ	%
250	245.8	3	2.95	98.3
250	245.8	3	2.95	98.3
500	493.7	6	5.90	98.7
500	495.8	6	5.95	99.2
625	626.7	7.5	7.52	100.2
625	626.7	7.5	7.52	100.2
750	766.7	9	9.2	102.2
750	747.5	9	8.97	99.7
1000	995.9	12	11.95	99.6
1000	1000.0	12	12.00	100.0
1250	1262.5	15	15.15	101.0
1250	1241.7	15	14.9	99.3
1250	1275.0	15	15.3	102.0

M = 99.9
Standard deviation 1.2

Table II. Nicotinamide Recoveries

Tissue	Amide Added × 1.008[a]	Amide Recovered as Nicotinic Acid	Amide as Nicotinic Acid in Aliquot		Recovery of Amide
				Found	
	γ	γ	γ	γ	%
Egg albumin	1260	1241.7	15.12	14.9	98.5
	1260	1245.9	15.12	14.95	98.9
	1260	1262.5	15.12	15.15	100.2
	1260	1233.4	15.12	14.8	97.9
Rat kidney	630	615	7.56	7.30	97.6
Rat heart	630	642	7.56	7.7	101.8
Rat liver	630	634.7	7.56	7.62	100.8
Beef liver	630	629.2	7.56	7.55	100.0
	945	945.9	11.35	11.36	100.1
	37.8	37.8	0.45	0.45	100.0
Rat liver	880	893	10.58	10.72	101.4
Niacin 250 Amide 630					

M = 99.75
Standard deviation 1.31

[a] Factor for conversion of nicotinamide to nicotinic acid.

bromide. The solution is stable for months when kept in a refrigerator.

p-Methylaminophenol sulfate, 5% solution in 0.1 *N* sulfuric acid, is purified by washing solid amine with ethyl alcohol.

Dibasic sodium phosphate, 10%.

Citric acid solution, 10%.

Pepsin, U.S.P.

Procedure. Two 10-ml. aliquots from the acid-pepsin digest are transferred to two 25-ml. volumetric flasks, and 6 ml. of *N* sodium hydroxide are added. This makes the digest solution 0.38 *N* alkali in strength, with a pH of about 12. The flask and its contents are now heated in a boiling water bath at 96° C. (approximately the boiling point at this elevation) for 1 hour. This hydrolyzes the nicotinamide diphosphopyridine nucleotide and triphosphopyridine nucleotide into nicotinic acid.

The values thus obtained are total nicotinic acid in the oxidized form; reduced nicotinic acid is not determined by cyanogen bromide method (*12*). After heating, excess alkalinity is partially neutralized by adding 5 ml. of 1 *N* hydrochloric acid. The remaining alkali is now utilized together with zinc sulfate for the precipitation of the protein-split products produced by pepsin action.

To the partially neutralized alkaline digest 2 ml. of 10% zinc sulfate are added, the volume is made up to 25 ml., mixed well, allowed to stand 10 minutes, and centrifuged, and the supernatant liquid is decanted. This is used for the analysis and must have a pH of 7.0 to 7.2 for a successful precipitation with zinc sulfate.

For color development 25 × 200 mm. test tubes are used, graduated at 25 ml. From each of the centrifugates sets of tubes are set up as shown:

Tube	Aliquot, Ml.	Buffer, Ml.	CNBr Reagent, Ml.	Amine Reagent, Ml.	H_2O, Ml.
1	0	3	4	14	4
2	0	3	4	14	4
3	3	0	0	0	22
4	3	0	0	0	22
5	3	3	4	14	1
6	3	3	4	14	1
7	3	3	4	0	0

Tubes 1 and 2 are controls on the reagents; tubes 3 and 4 are controls on the color in the aliquot. The authors have not found any color to develop between unknown substances in the digest and the amine reagent (*5*). Tubes 5 and 6 are for the analysis, while tube 7 is set up to control the pH before the cyanogen bromide is added to tubes 5 and 6. If the pH is higher than 5.1 in tube 7 as determined by a glass electrode pH meter, a drop or two of 10% citric acid solution is added to tubes 5 and 6; if below 5.1, a drop or two of 10% dibasic sodium phosphate solution is added.

After pH correction, the cyanogen bromide is added to the tubes and is allowed to react in the dark for 30 minutes at 25° C. The tubes are then cooled to 10° to 15° C. and 14 ml. of cooled 5% *p*-methylaminophenol sulfate reagent are added. The samples are made up to volume and the tubes are allowed to stand in the dark at 10° to 15° C. for 80 minutes.

Density readings are taken, a Coleman Universal spectrophotometer being used and set at a wave length of 410 mμ. Distilled water is used as a reference sample in setting the machine. For calculation, the average density reading of tubes 1 and 2 is added to that of tubes 3 and 4 and the sum is subtracted from the average density reading of assay tubes 5 and 6. The values obtained are converted into nicotinic acid values either by use of a concentration reference curve or by calculation from a K value determined from known amounts of the acid carried through the procedure.

As nicotinic acid may be present in the pepsin preparations used, an analysis should be carried out on the material and a proper correction made if necessary. None was found in the Merck's pepsin preparations used in these studies.

Table III. Nicotinic Acid Values in Male Rat Tissues

(Comparison with values obtained by other chemical and microbiological procedures)

Tissue	Age of Rats	Present[a] Method	Dann and Handler Authors'[a] analysis	Dann and Handler Original[b] report	Singal[c] *et al.*
	Days	γ/g.	γ/g.	γ/g.	γ/g.
Liver	24	...	...	159 (148–176)	...
	45–55	172 (159–183)	143 (123–171)	...	...
	60–72	146 (138–152)	137 (132–144)	...	...
	91–94	...	...	...	157 (130–173)
	135	148 (147–156)	...	...	...
	270	144 (127–157)	...	175 (151–191)	...
Kidney cortex	24	...	...	115 (98–122)	...
Kidney whole	60–72	144 (98–123)	95 (79–106)	...	...
	91–94	...	...	...	87 (80–92)
	135	110 (107–114)	...	...	...
	270	87 (77–112)	...	...	...
Kidney cortex	270	...	...	132 (112–151)	...
Heart	90	...	...	...	114 (108–119)
	135	92 (70–109)	...	...	...
	270	80 (47–120)	...	...	...
Muscle					
Leg	24	...	...	77 (63–87)	...
Abdominal	60–72	62 (55–73)	52 (39–62)	...	...
	90	...	...	...	78 (73–84)
	135	56 (53–61)	...	...	...
Leg	270			86 (81–92)	...
Abdominal	270	54 (40–62)	...	...	...

[a] Sprague-Dawley strain of rats.
[b] Dann and Handler CNBr procedure (*3*), Vanderbilt strain of rats.
[c] Singal *et al.* microbiological values (*12*), Wistar strain of rats.

RECOVERY EXPERIMENTS

Recoveries of nicotinic acid added to egg albumin are shown in Table I, and added nicotinamide recoveries from egg albumin and tissue digests are shown in Table II. The range of nicotinic acid recovery was from 98.3 to 102.2% with a mean of 99.9% and a standard deviation of 1.2%. The range of nicotinamide recoveries was from 97.6 to 101.8% with a mean of 99.75% and a standard deviation of 1.31%. As shown in the last line in Table II,

recoveries were good where both nicotinamide and nicotinic acid were added.

In Table III a comparison is made of values for aliquots of the same rat tissues, using the present procedure and that of Dann and Handler (*3*). For additional comparison the values originally reported by Dann and Handler (*3*) are given, as well as those obtained by Singal *et al.* (*12*) using a microbiological procedure (*13*).

It is the finding of this laboratory that many factors enter into the amounts of nicotinic acid and its derivatives which are found in tissues. Age, the amount of tryptophan in the diet, and the strain of rat must be considered when comparing values reported from different laboratories. When the Dann and Handler method and the present procedure were used on the same tissues, higher values were obtained by the latter. The microbiological procedure would be expected to give higher values, as the values obtained by the cyanogen bromide procedures do not include any reduced diphosphopyridine nucleotide present in tissues. The cyanogen bromide reaction does not take place where the α-carbon in nicotinic acid is substituted (*14*). In spite of this, the values of Singal *et al.* fall in the same range.

Excellent recoveries of nicotinic acid in egg albumin digest were obtained when only 3 micrograms were present (Table I). In other recovery experiments values on the order of 1 microgram of nicotinic acid per final aliquot were determined quantitatively with an accuracy of 98%. Table II shows that 0.45 microgram of nicotinamide added per aliquot was quantitatively recovered. These recoveries show the applicability of the procedure for the accurate determination of small amounts of nicotinic acid and its derivatives. Trigonelline was not converted into nicotinic acid by this procedure (*11*). Nicotinuric acid was not tried. Nicotinic acid has been estimated in various ingredients of a rat's diet. Dextrin gave no nicotinic acid, zein contained 791 micrograms per 100 grams, while celluflour contained 825 micrograms per 100 grams. Using a diphosphopyridine nucleotide preparation obtained from the Schwarz Laboratories, New York, which was rated at a 60% diphosphopyridine nucleotide content, the authors found values of 58.2 and 59.1%. The procedure as described appears applicable, therefore, to the accurate determination of nicotinic acid plus diphosphopyridine nucleotide in a wide variety of plant and animal products.

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Stable Isotope Dilution Method for Nicotinic Acid Determination

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The application of the isotope dilution technique to the determination of nicotinic acid was developed because it became necessary to assay accurately for the nicotinic acid content of crude products known to contain substantial amounts of other compounds containing pyridine rings, and because a review of the literature revealed the existence of no analytical method which would not be seriously affected by the presence of such impurities. A stable isotope dilution method is described in which deuteronicotinic acid is used as the tracer and its dilution is determined by infrared spectrophotometry. The method provides an absolute assay for nicotinic acid in complex mixtures, and has the general significance for analytical chemistry of further illustrating the importance of the isotope dilution principle in the development of specific (absolute) assay methods, a field which has been neglected in the past.

EVER since the discovery (*4*) of the vitamin activity of nicotinic acid in 1937, there has been considerable interest in methods for its determination, especially in complex mixtures. As a result there has appeared in the literature a relatively large number of papers describing such assay methods. No attempt is made here to review these methods, as adequate bibliographies are available in a number of places (*1, 3*).

In general, these methods of determination may be divided into two types, microbiological and chemical. Microbiological methods are characterized by relatively good specificity, but are most difficult to carry out with any high degree of precision. A great many papers have been written describing chemical methods of assay, but in every case the fundamental reaction involved is the same—that is, the Konig reaction (*7, 8*) in which the pyridine ring is opened by reaction with cyanogen bromide to give a product which is capable of further reaction with a primary or secondary amine to give a yellow colored product, the amount of which may be determined spectrophotometrically. Because many pyridine ring-containing substances will give this same reaction, such a method cannot be very specific and this fact is amply borne out by experience (*2*). Under these circumstances and because the materials for which assays were required were known to contain closely related pyridine-ring compounds (6-methyl nicotinic acid), the authors again (*11*) turned to the method of isotope dilution as one which would not be subject to any of the above disadvantages.

A detailed discussion of the general nature of the authors' particular methods of application of the stable isotope dilution principle has been presented elsewhere (*10, 11*). Briefly, the present method of assay is based upon the addition of a known amount of deuteronicotinic acid to a known amount of the sample to be assayed, followed by esterification of the whole mixture and qualitative isolation of a pure specimen of the mixed (isotopically) methyl nicotinates. The analysis is completed by determination of the ratio of methyl protionicotinate to methyl deuteronicotinate in the isolated specimen by direct infrared spectrophotometry. In the course of developing this assay procedure, the solubility properties of nicotinic acid were so unfavorable that no suitable solvent could be found in which the necessary infrared spectrophotometric measurements could be made. Attention was then turned to the methyl ester as possessing more favorable solubility properties. This proved to be the case and it was found that an additional advantage accrued through its use in that it could be more readily purified than the acid, a property vital to the ultimate reliability of any isotope dilution assay (*10*). The low melting point of methyl nicotinate, moreover, proved a sensitive criterion of purity of the isolated specimen on which the spectrophotometric measurement of isotope dilution was made.

The method of isotope dilution, when applied for the purpose of chemical analysis, suffers from the disadvantage that it is necessary to isolate qualitatively a pure specimen of the substance for which assay is required. Generally, this can seldom be achieved with less than approximately 1 mg. of the sought substance. As a consequence, the method becomes progressively more difficult to apply to solid specimens which contain only small amounts of the compound for which assay is sought, unless some simple and selective process is known whereby the latter may be concentrated in relatively pure form. In many instances, therefore, the application of the isotope dilution method will not be practical as a general analytical method for everyday use. It will, nevertheless, be the method of choice when it is of paramount importance to obtain a completely reliable assay or when it is essential to check the reliability of some other more convenient but less certain assay method. In other words, the need for reliability must be sufficiently great to justify the additional expenditure in time and effort which may be required in order to achieve isolation at the necessary level. Thus, modern micromanipulation techniques are of considerable value in rendering more feasible the achievement of the desired microscale isolations.

PREPARATION OF DEUTERONICOTINIC ACID AND ITS METHYL ESTER

Deuterization of nicotinic acid was effected by direct exchange using deuterosulfuric acid similar to that used with benzene (*5*).

Deuterosulfuric acid (80%) was prepared in an all-glass, water-cooled apparatus equipped with a magnetic stirrer, by the addition of the required amount of sulfur trioxide (Baker and Adamson Sulfan B) to 99.8% deuterium oxide (Stuart Oxygen Co.).

Four grams of pure nicotinic acid and 25 ml. of 80 weight % pure deuterosulfuric acid in pure deuterium oxide were heated at 300° C. in a sealed Carius tube for 90 to 120 hours. After cooling to room temperature, the Carius tube was opened, the contents were transferred with water washing to a 250-ml. Erlenmeyer flask immersed in an ice bath, and 60 ml. of 30% sodium hydroxide solution were added slowly with stirring. Finally, the pH was adjusted to between 2 and 3 and the aqueous solution was extracted continuously for 24 hours with 200 ml. of ethyl acetate. After evaporation of the ethyl acetate extract to dryness, the residue was picked up in a minimum volume of boiling water, charcoaled lightly to remove color, filtered hot, and allowed to crystallize on cooling. Recrystallization was effected by solution in a minimum volume of hot absolute ethyl alcohol, hot filtration, and cooling.

In this manner an 83% yield of deuteronicotinic acid was obtained. The deuteronicotinic acid was characterized by the following properties: observed melting point 234° to 235.5° C.; melting point of nicotinic acid 234° to 236° C; composition of $C_6H_2D_3O_2N$, analytically calculated: carbon, 57.2, nitrogen, 11.1; composition found: carbon, 57.6, nitrogen, 11.3; ultraviolet absorption spectrum in 95% ethyl alcohol: pure nicotinic acid, molecular extinction coefficient, E_M, 2900 at 2630 A.; deuteronicotinic acid, E_M 2920 at 2630 A.

The atom per cent of deuterium was determined by combustion of the deuteronicotinic acid to water, the deuterium content of which was determined by the falling drop method (*6*). The results indicated that the deuteronicotinic acid contained 54.7 ± 3 atom % of deuterium as the average of two determinations. For three atoms of deuterium in nicotinic acid, the atom per cent would be 60; thus, on the average, three quarters of the total number of nonexchangeable hydrogens have been exchanged in the tracer material, which proved sufficient to give the analytically required discriminatory properties in the infrared absorption spectra of the two isotopic analogs.

The preparation of the methyl ester was carried out by treating each gram of deuteronicotinic acid with 10 ml. of methanol and 1.5 ml. of fuming sulfuric acid (20% free sulfur trioxide) followed by refluxing on a steam bath for 1 hour. After transferring the reaction mixture to a separatory funnel and adding 20 ml. of a standard neutralizing reagent (115 ml. of 6*N* ammonium hydroxide and 60 grams of ammonium sulfate in a 200-ml. total volume), the whole was twice extracted with two 100-ml. portions of petroleum ether. The petroleum ether extracts, after combination, were concentrated to about 20 ml. or less, charcoaled with about 0.5 gram of Darco G60, and filtered and washed with a little petroleum ether; the total volume of the filtrate did not exceed 28 ml. On chilling the petroleum ether filtrate to 0° C. for 2 hours, white crystals formed which were isolated by cold filtration and dried in vacuo at room temperature for 0.5 hour. The melting point should be 41° to 42° C. If the melting point is lower, the crystallization procedure should be repeated.

In this manner a 70% yield of pure methyl ester was obtained.

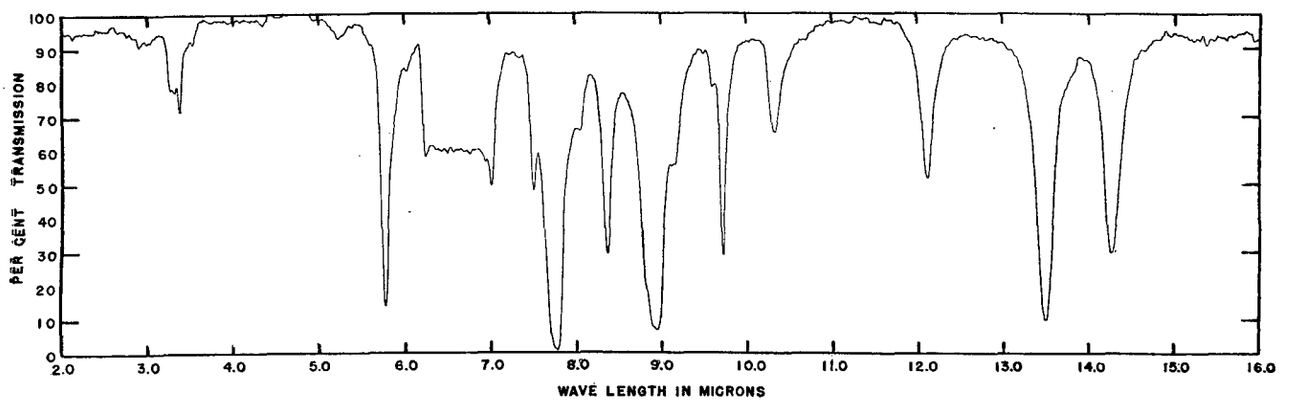

Figure 1. Methyl Nicotinate in Carbon Disulfide Solution

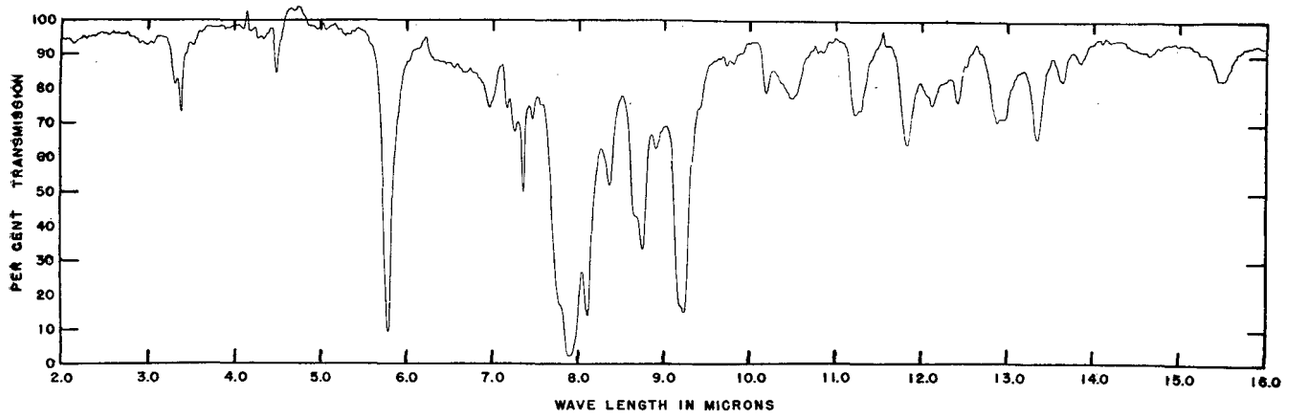

Figure 2. Methyl Trideuteronicotinate in Carbon Disulfide Solution

In addition to the melting point, the product was characterized by its ultraviolet absorption and was found to give results identical to those observed with an authentic sample of pure methyl nicotinate—E_M 3020 at 2630 A.

SPECTROPHOTOMETRIC DETERMINATION OF ISOTOPIC DILUTION

A superficial comparison of the spectra in Figures 1 and 2 reveals a situation almost ideal for the spectrophotometric determination of the individual isotopic analogs in mixtures of the two. The introduction of deuterium into the pyridine ring of methyl nicotinate profoundly changes its infrared absorption spectrum. Particular attention is called to 8.95, 9.75, 12.10, 13.50, and 14.27 mu bands of the protio analog and to the 4.50, 7.37, 8.12, 9.22, and 11.21 mu bands of the deutero analog, any one of which could be used to determine the ratio of methyl protionicotinate to methyl deuteronicotinate in a mixture of the two. The authors found the 13.50 mu band of methyl protionicotinate, a region in which the deutero analog is transparent, the most favorable for analytical use and consequently all subsequent work was carried out at this wave length.

The spectrophotometric techniques used in this work have been improved over those reported in the authors' earlier publication (*11*). As in the earlier work, a carefully calibrated (*9*) Model 12A Perkin-Elmer infrared spectrometer was used. All measurements were carried out with the instrument set at 13.50 mu; the globar was operated with a power input of 125 watts, an amplifier gain corresponding to 1 microvolt full scale on the standard Brown recorder chart, and slits of 0.900 mm. A sealed liquid cell with rock salt windows of 0.10-mm. path length was used for the unknowns solution. For a reference state the authors found the use of a 0.20-mm. cell containing pure liquid benzene best.

Such a system had close to the same transmittance at 13.50 mu as the 0.10-mm. cell when it is filled with a carbon disulfide solution at a total concentration of 50 mg. per ml. of a 50 to 50 mixture of methyl protionicotinate and methyl deuteronicotinate. The chief advantage of such a reference state is its complete reproducibility over long periods of time in contrast with a standard solution (50 mg. per ml. of a 50 to 50 mixture of the two isotopic nicotinates in carbon disulfide), which may change its concentration on storage and handling. Any changes in the constants of the reference cell employed in this work were guarded against by daily check of its apparent transmittance, when filled with benzene, relative to a selected 16-mesh wire screen. In similar manner the benzene-filled unknowns cell was checked against the benzene-filled reference cell. This checking procedure, based on a stable standard, benzene, constitutes in effect a check of the reliability of the established calibration curve; it was found entirely satisfactory over the long period of time during which this technique was tested by repeated calibration-curve checks with solutions of known composition. The transmittance values were always determined in terms of a 0% line set with the lithium fluoride filter in the incident beam to minimize scattered radiation effects.

The actual calibration curve was established by successively filling the unknowns cell (0.10 mm. in length) with carbon disulfide solutions of fixed total concentration (50 mg. per ml.), but of various known ratios of methyl protionicotinate and methyl deuteronicotinate, and determining their apparent absorbance relative to the benzene-filled reference cell (0.20 mm. in length). The data are given in Table I. The carbon disulfide solutions were always made up by weighing on a microbalance a known amount of the sample to be examined in a glass-stoppered Erlenmeyer microflask (about 2- to 3-ml. volume) to eliminate sublimation of the relatively volatile methyl nicotinates; then the required volume of carbon disulfide was added from a 1-ml. microburet calibrated in 0.001 ml. in order to give a solution of standard concentration—that is, 50 mg. per ml. of solvent. Solution was effected in the stoppered flask and could be checked for correct amount of solvent by back-weighing if necessary. The solution was introduced into the unknown cell by means of a hypodermic syringe; the cell was placed in the spectrometer and its transmission was measured under the spectrometer settings indicated earlier. Immediately the unknown cell was replaced by the benzene-filled reference cell and its transmittance was also determined. This was repeated alternately until a satisfactorily constant transmission ratio (transmittance), within 0.006 unit, is obtained.

Table I. Calibration Data for Methyl Protionicotinate–Methyl Deuteronicotinate Mixtures

Deutero Component, %	Absorbance, 13.50 mu (Av. of 2 Detns.)
33.6	−0.074
45.4	+0.004
59.8	+0.102
68.5	+0.162

Generally only two such cell interchanges are required to establish a satisfactory ratio from which the deutero-protio composition of an unknown can be evaluated by means of the calibration curve or the calibration equation.

Beer's law is followed closely; the data in Table I may be represented by the following straight-line equation:

$$\log R = d = 0.680\,P_T - 0.305 \qquad (1)$$

where R is the ratio of the transmission of the unknown solution to the transmission of the benzene reference standard, d is the absorbance, and P_T is the per cent of the deutero component in the sample under analysis.

In order to preserve maximum precision in an isotope dilution assay, it is desirable to add an amount of tracer to the unknown as nearly equivalent as possible to the amount of substance whose assay is sought (*10*, *11*); thus, it is not essential to extend the calibration much beyond the composition limits given in Table I.

Table II. Spectrophotometric Reproducibility

Date	P_T	Deviation
12-22-49	44.9	−0.3
12-23-49	44.9	−0.3
1-3-50	45.5	+0.3
1-17-50	45.0	−0.2
1-20-50	45.3	+0.1
2-2-50	45.2	0.0
	Av. 45.1	±0.2

ISOTOPE DILUTION ASSAY PROCEDURE

A specimen of the sample to be assayed is weighed out exactly (W_s); the size of the sample is such that it will contain roughly 100 mg. of nicotinic acid, to which are added about 100 mg. (exact weight W_T) of the deuteronicotinic acid tracer. The mixture is placed in a small (2- to 3-ml.) round-bottomed flask, 1.5 ml. of methanol and 0.3 ml. fuming (20% free sulfur trioxide) sulfuric acid are added, and the whole is refluxed for 1 hour on the steam bath. After transferring the crude methyl nicotinates to a 25-ml. glass-stoppered cylinder (graduated), containing 4 ml. of the standard neutralizing reagent (115 ml. of 6 N ammonium hydroxide and 60 grams of ammonium sulfate in a 200-ml. total volume), 15 to 20 ml. of petroleum ether are added and the whole is brought to equilibrium by vigorous shaking. After separation of the phases, a second 15- to 25-ml. portion of petroleum ether is added and the extraction is repeated. The combined petroleum ether extracts are evaporated to about 5 ml. or less. Fifty milligrams of Darco G-60 are added, the solution is filtered, and the charcoal is washed with a little petroleum ether, keeping the total filtrate volume below 7 ml. (evaporate if necessary). The methyl nicotinates are crystallized by chilling to 0° C. for 2 hours, filtering cold, and drying under vacuum at room temperature for 0.5 hour. The melting point must be sharp at 41° to 42° C.; if it is lower, the crystallization procedure must be repeated.

When a satisfactorily pure specimen has been isolated, as judged by melting point, the per cent of the deutero component in the sample, P_T, is determined spectrophotometrically. This procedure works well with the kinds of specimens the authors have assayed. In special cases a modified isolation procedure may be required because of the nature of the specimen to be assayed, but the petroleum ether extraction step seems very effective in separating methyl nicotinate from other substances. Operating with the quantities indicated, yields varying between 50 and 100 mg. were obtained. In preparing the carbon disulfide solutions for infrared measurement, a 25.0-mg. portion was generally weighed out and dissolved in 0.500 ml. of the solvent. Thus, all the quantities are sufficiently large to render all manipulations comfortable. If smaller samples are used, the problem can easily be met through the use of microabsorption cells which are now available from the Perkin-Elmer Corp. These cells may be obtained with a path length of 3 mm. which makes possible the use of solutions of only 1 mg. per ml. concentration and, moreover, less than 0.1 ml. of solution is required to fill them. Thus, samples as small as 0.1 mg. may be analyzed for tracer dilution; this would reduce the required sample size by at least a factor of 100, relative to the amounts the authors used.

COMPUTATION OF RESULTS

Like any isotope dilution assay, and as described in detail in an earlier paper (*11*), the desired numerical result is computed from the following equation:

$$F = \frac{W_T (1 - P_T)}{W_s P_T} \times 100 \qquad (2)$$

where F = weight per cent of nicotinic acid in sample
P_T = per cent of deutero component in sample
W_s = weight in milligrams of sample taken
W_T = weight in milligrams of tracer added to W_s

Table II presents data illustrating the reproducibility of infrared spectrophotometric measurements as a function of time. All measurements were made with a standard mixture containing 45.2% methyl deuteronicotinate and 54.8% methyl protionicotinate.

Table III presents data illustrating the reliability of the over-all assay procedure including isolation. Known mixtures were assayed as though they were unknowns with results as in Table III.

The data in Table III clearly reveal the absence in the isolation procedure of any significant degree of fractionation of the isotopic analogs.

Table IV presents a complete summary of the results obtained using the techniques described herein with actual unknowns.

All these results led the authors to evaluate the average precision as at least ±1%; inasmuch as precision and reliability are equal (*11*), the authors believe that the assays are within ±1% of the absolute amount of nicotinic acid in the unknowns studied.

DISCUSSION

The general factors which play a role in the spectrophotometric–isotope dilution assay technique described herein have been discussed in earlier publications (*10, 11*). Some of the generalities, only envisaged then, have now been successfully reduced to practice, a justification of the broad scope claimed for this approach to the analysis of specific organic entities in complex media.

ACKNOWLEDGMENT

The authors wish to express their sincere thanks to R. N. Boos and his colleagues for the ultimate analyses and to L. J. Wissow for his help in preparing some of the pyridine carboxylic acids used in this investigation.

Table III. Over-all Reproducibility

P_T, As Made Up	P_T, Found	Deviation
44.4	44.8	+0.4
49.6	48.7	−0.9
33.7	33.7	0.0
60.2	60.6	+0.4

Table IV. Analysis of Crude Nicotinic Acids

Sample	Nicotinic Acid, Wt. % (F)
1	48.4, 49.5
2	36.0, 34.5
3	42.3, 42.7
4	44.2, 43.6
5	72.7, 72.7, 71.4
6	66.2, 66.5
7	52.3, 53.7
8	61.9, 64.3, 64.3, 64.0
9	66.2, 64.1

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Tris(hydroxymethyl)aminomethane as an Acidimetric Standard

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The need for a good acidimetric standard suggested investigation of the properties of tris(hydroxymethyl)aminomethane. It was prepared in a state of high purity with constant composition. Its hygroscopicity was comparable to that of potassium hydrogen phthalate; neither it nor its solutions adsorb carbon dioxide from the air; it can be dried by heating at 100° to 103° C.; its solutions are stable under all investigated conditions of standardization; the pH of its equivalence point is 4.7; and *p*-sulfo-*o*-methoxybenzeneazodimethyl-1-naphthylamine is the best indicator for determining the equivalence point. Tris(hydroxymethyl)aminomethane has many properties of an ideal primary standard. It is easily obtained, purified, dried, and preserved in a pure state, has low hygroscopicity and relatively high equivalent weight, undergoes stoichiometric reaction, and has a negligible indicator blank.

THERE is an apparent need for a good primary-type standard for the standardization of dilute solutions of strong acids. Most substances now in use fail to meet the requirements of a good standard because they have a low equivalent weight, are difficult to prepare and maintain in a high state of purity, or are hydrates or hygroscopic. The purpose of this paper is to suggest the use of tris(hydroxymethyl)aminomethane for the standardization of dilute solutions of strong acids.

To establish a material as a primary standard, the work of several independent workers or groups is required. By suggesting the use of tris(hydroxymethyl)aminomethane as a standard for strong acids, the authors hope to create sufficient interest so that the material will be further tested by methods other than those presented in this paper. For this reason the methods used in the present investigation are described in some detail. Typical data are presented to indicate the accuracy obtained by the authors.

EQUIPMENT

Volumetric 50-ml. buret, calibrated by National Bureau of Standards.

pH Meter, Leeds and Northrup No. 7661-A1 assembly.

REAGENTS

Hydrochloric Acid Solution, 0.1 *N*, prepared from reagent grade concentrated hydrochloric acid.

Sodium Hydroxide, 0.1 *N*, prepared from a filtered 50% solution of C.P. sodium hydroxide.

Tris(hydroxymethyl)aminomethane, purified as described below.

Potassium Hydrogen Phthalate, National Bureau of Standards standard sample 84c, effective neutralizing power 100.05%.

Sodium Carbonate, purified by the method of Waldbauer, McCann, and Tuleen (*5*).

p-Sulfo-*o*-Methoxybenzeneazodimethyl-1-Naphthylamine Indicator Solution. Triturate 100 mg. of the indicator in a mortar with 2.59 ml. of 0.1 *N* sodium hydroxide solution and dilute to 100 ml. with boiled distilled water.

Ethyl Orange Indicator Solution. Dissolve 100 mg. of the indicator in 100 ml. of boiled distilled water.

Mixed Indicator. Mix in equal parts 100 mg. of bromocresol green triturated in a mortar with 1.45 ml. of 0.1 *N* sodium hydroxide and diluted to 100 ml. with boiled distilled water, and 100 mg. of alizarin red S (sodium alizarin sulfonate) dissolved in 100 ml. of boiled distilled water.

Reference Buffer Solution of pH 4.70 for colorimetric determination of end point. Mix 50 ml. of 0.1 *N* potassium hydrogen phthalate solution, 13.1 ml. of 0.1 *N* sodium hydroxide solution, 3 drops of the appropriate indicator solution or 6 drops of the mixed indicator, and 25 ml. of boiled distilled water.

PURIFICATION OF TRIS(HYDROXYMETHYL)AMINOMETHANE

Dissolve 1200 grams of tris(hydroxymethyl)aminomethane in 2400 ml. of distilled water in a 4-liter beaker by heating the mixture to 60° C. Add 50 grams of Norite and keep the mixture at 50° to 60° C. for 30 minutes with constant stirring. Filter the mixture, while hot, through a fritted-glass filter funnel of fine porosity, containing a 0.6-cm. (0.25-inch) mat of filter pulp, into a 4-liter suction flask to remove the Norite. Repeat the treatment. The filtrate should be nearly colorless.

Transfer the solution to a 4-liter beaker, add 2 or 3 glass beads to prevent bumping, cover with a ribbed watch glass, and concentrate, at a slow boil, to a volume of about 1300 ml. Equip two 4-liter beakers with mechanical stirrers and add 2000 ml. of purified methanol to each. Add half of the concentrated solution to each portion of methanol by pouring the solution slowly down the side of the beaker. Continuously stir the methanol during this addition and during the crystallization period. Allow the mixture to cool for about 30 minutes, then place the beakers in an ice-salt-water slurry and cool the mixture to 3° to 4° C. Remove the mother liquor by filtration through a fritted-glass filter funnel of coarse porosity. Wash the product once on the filter by slurrying with cold methanol and remove the surplus methanol by rapid suction.

Recrystallize twice more, using the same technique but omitting the Norite treatment. For the second recrystallization, dissolve the amine in 1000 ml. of redistilled water, concentrate to a total volume of 1000 ml., and pour into 3000 ml. of methanol. For the third recrystallization, dissolve the amine in 800 ml. of redistilled water, concentrate to a total volume of 800 ml., and pour into 2500 ml. of purified methanol. Wash the product obtained from the third recrystallization in the funnel by slurrying twice with cold methanol. Air-dry the final product between several sheets of filter paper.

Grind the air-dried product to pass a 50-mesh screen. Place the screened amine in Petri dishes to a depth of 0.6 cm. and dry in a vacuum oven for 12 hours at 60° C. at not more than 10 to 15 mm. pressure, or, preferably, in a vacuum desiccator over phosphorus pentoxide for 24 to 36 hours at not over 5-mm. pressure, maintaining the vacuum during the entire drying period. The dried tris(hydroxymethyl)aminomethane melts at 171.1° ± 0.2° C. (corrected) when heated at a rate of 6° C. per minute.

The methanol used in purification of the tris(hydroxymethyl)-aminomethane in this investigation was Commercial Solvents Corp. commercial grade methanol purified by distillation through a 25-plate Penn State type column at a reflux ratio of about 1 to 1.

The water used for the second and third recrystallizations was distilled from alkaline permanganate. The water still was provided with an adequate spray trap.

COMPARISON OF STANDARDS

Tris(hydroxymethyl)aminomethane was compared with two commonly used standards (*2*), sodium hydroxide solution and sodium carbonate, by standardizing a hydrochloric acid solution with each.

The sodium hydroxide solution was standardized potentiometrically with potassium hydrogen phthalate, National Bureau of Standards standard sample 84c. The hydrochloric acid solution was standardized with the standard sodium hydroxide solution potentiometrically, and with sodium carbonate according to the method of Kolthoff and Sandell (*4*).

The standardization with tris(hydroxymethyl)aminomethane

Table I. Standardization of Solutions

Standard	$HKC_8H_4O_4$	NaOH	Na_2CO_3	$(CH_2OH)_3CNH_2$
	Sodium Hydroxide, *N*	Hydrochloric Acid, *N*		
	0.10127	0.10075	0.10093	0.10080
	0.10126	0.10075	0.10084	0.10072
	0.10129	0.10068	0.10084	0.10073
	0.10130	0.10071	0.10088	0.10076
	0.10131	0.10071	0.10093	0.10077
	0.10131		0.10088	
Mean	0.10129	0.10072	0.10088	0.10076
Mean dev.	0.000017	0.00002	0.00003	0.00002

was carried out by the following procedure: Approximately 0.5 gram of the amine was accurately weighed into a 250-ml. tall-form beaker. The sample was dissolved in 50 ml. of recently boiled distilled water and titrated potentiometrically with the hydrochloric acid solution.

The results of standardizations are given in Table I.

The pH at the equivalence point of tris(hydroxymethyl)-aminomethane and hydrochloric acid under the conditions of standardization was determined mathematically and graphically to be 4.70. The neutralization curve of the amine and hydrochloric acid shows a clean sharp break at the equivalence point.

All standard solutions were corrected for change in volume due to temperature change.

Table II. Purity of Tris(hydroxymethyl)aminomethane as Determined with Several Indicators

	Potentiometrically, %	SMN[a] Indicator, %	Ethyl Orange Indicator, %	Mixed Indicator, %
	99.92[b]	99.95	99.98	99.96
	100.00	99.92	100.04	99.93
	99.99	99.97	99.94	99.93
	99.96	100.00	100.02	
	99.95	99.91	99.97	
Mean	99.96	99.95	99.99	99.94

[a] *p*-Sulfo-*o*-methoxybenzeneazodimethyl-1-naphthylamine.
[b] Calculated from data in Table I.

SELECTION OF INDICATORS

Several indicators whose pH range included the equivalence point of the titration were selected for test. The indicators were screened by adding a few drops of their solution to a solution of the amine, and titrating potentiometrically with hydrochloric acid. Three indicators, *p*-sulfo-*o*-methoxybenzeneazodimethyl-1-naphthylamine, ethyl orange, and a mixed indicator consisting of equal parts of 0.1% solutions of bromocresol green and sodium alizarin sulfonate, gave a distinctive color at a pH of 4.70.

The three indicators selected from the screening tests were evaluated as follows:

Tris(hydroxymethyl)aminomethane (0.5 gram) was accurately weighed into a 250-ml. Erlenmeyer flask and dissolved in 50 ml. of recently boiled distilled water. Three drops of the indicator solution (6 drops of the mixed indicator) were added and the amine was titrated with standard hydrochloric acid. The end point of the titration was determined by matching with the color of the reference buffer solution. The strength of the standard acid was 0.10072 *N* (Table I).

The results given in Table II were obtained.

p-Sulfo-*o*-methoxybenzeneazodimethyl-1-naphthylamine is preferred because it gives the sharpest color change at the equivalence point and because it gives good warning as the end point is approached.

THERMAL STABILITY

The stability of tris(hydroxymethyl)aminomethane was determined at room and at elevated temperatures.

A sample of tris(hydroxymethyl)aminomethane was kept on the laboratory shelf for 2 years in a screw-cap jar firmly closed with a metal cap containing a plastic-impregnated liner. At the end of the test period there was no detectable change in composition.

Three portions of the amine were stored in a desiccator for 3 days over phosphorus pentoxide, and weighed each day. No change in weight was detected.

Portions of the amine, heated in a laboratory drying oven at 103° C. for five 2-hour periods, were weighed at the end of each heating period. A large sample was treated in the same manner and analyzed potentiometrically with hydrochloric acid after each heating period. Typical results are given in Table III.

Duplicate titrations were run only on the 0-hour and 10-hour material of sample 4. As the analysis indicates the purity remained constant, the loss of weight may be due to partial volatilization of the compound.

A sample was heated at 110° C. for 10 hours, weighed, and analyzed. The material after heating was a light cream color speckled with small brown spots.

The data in Table III indicate that the amine can be dried for 2 to 4 hours at 100° to 103° C.

HYGROSCOPICITY

The hygroscopicities were determined for tris(hydroxymethyl)-aminomethane and several materials commonly used as standards. The sample of tris(hydroxymethyl)aminomethane was the same material used for the titrimetric studies.

Mallinckrodt's primary standard grade potassium hydrogen phthalate and A.C.S. reagent grade potassium chloride were recrystallized twice from conductivity-type water. The salts were dried, ground to pass a 50-mesh screen, redried in an oven overnight at 105° C., and stored in a vacuum desiccator over phosphorus pentoxide in vacuo until ready for use.

Mallinckrodt's primary standard grade benzoic acid was recrystallized twice from neutral 95% ethyl alcohol which had recently been fractionally distilled. It was dried, ground to pass a 50-mesh screen, redried, and stored in a vacuum desiccator over phosphorus pentoxide in vacuo until ready to use.

The hygroscopicities were determined at 25° ± 0.5° C. in desiccators containing a salt in contact with its saturated solution as described in the International Critical Tables (*3*).

The sample containers used for determining the hygroscopicities (30 × 50 mm. weighing bottles of 30-ml. capacity) were thoroughly cleaned, dried, and placed overnight in the desiccator containing the appropriate humidity. During all periods of exposure in the humidity chambers, the covers of the weighing bottles were placed in a manner to permit circulation of air in the sample container. The weighing bottles were removed from the desiccator, immediately stoppered, allowed to stand in air 10 ± 1 minutes, and weighed. Approximately 1-gram samples of the materials being studied were placed in the weighing bottles, and spread to a uniform thickness. The weighing bottles were

Table III. Stability of Tris(hydroxymethyl)aminomethane at 103° C.

(Sample weight 2.0150 grams)

Sample No.	Heating Time, Hours	Weight Loss, Mg.	Weight Loss, %	Analysis, %
	2	0.8	0.040	...
	4	1.5	0.074	...
	6	2.3	0.114	...
	8	2.5	0.124	...
	10	3.2	0.159	...
	0	...	...	99.95
	2	...	...	99.95
	4	...	...	99.99
	6	...	...	99.94
	8	...	...	99.98
	10	...	...	99.94

Table IV. Stability of Tris(hydroxymethyl)aminomethane at 110° C.

Heating Period, Hours	Weight of Sample, Grams	Analysis, %
0	2.0409	99.95
10	2.0237	98.82

Table V. Hygroscopicity of Several Standards

(10-day tests)

Relative Humidity, %	Gain in Weight, %			
	Tris(hydroxymethyl)-aminomethane[a]	Potassium hydrogen phthalate[b]	Benzoic acid	Potassium chloride
31	0.19	0.06	0.00	0.08
51	0.17	0.07	0.00	0.13
71.2	0.19	0.17	0.00	0.14
91	0.27	0.20	0.01	0.47

[a] Average of 3 determinations.
[b] Average of 2 determinations.

stoppered and weighed, then placed in the proper desiccator, and placed in the thermostat. These bottles were weighed daily for 10 days, using the technique described for taring the weighing bottles. Representative data are shown in Table V.

The hygroscopicity tests were carried out in May, during which time the air temperature and humidity varied widely. The adsorbed moisture on the weighing bottles is the cause of the major error in the low adsorption range. Errors as high as 0.5 mg. on unfilled weighing bottles were not uncommon when these bottles were weighed on days of widely varying humidity.

The hygroscopicity of tris(hydroxymethyl)aminomethane compares favorably with that of the common primary standards which were tested over all humidity ranges that might be encountered in laboratory work.

CONCLUSION

Tris(hydroxymethyl)aminomethane fulfills many of the requirements of a good standard. It is commercially available at a moderate price; it can be prepared in a high state of purity with constant composition; and it has a favorable equivalent weight, 121.136. The amine is nonhygroscopic at usual laboratory humidities and compares favorably in this respect with potassium hydrogen phthalate. Tris(hydroxymethyl)aminomethane and solutions of this salt do not adsorb carbon dioxide from the air. It can be used directly as a primary standard for strong acid solutions by a simple and rapid procedure. Solutions of the amine are stable under all investigated conditions of standardization. The equivalence point can be easily detected either potentiometrically or by use of the proper indicator.

Tris(hydroxymethyl)aminomethane has the disadvantage that it cannot be heated above 100° C. indiscriminately. It is a weak base and has the inherent disadvantage of this class of compounds as primary standards. Its dissociation constant has been reported recently by Bates and Pinching (*1*) as 1.202×10^{-6} at 25° C. This value is comparable to that of potassium hydrogen phthalate, which is 3.9×10^{-6} at 18° C.

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Determination of *n*-Paraffins in Gasoline, Oils, and Paraffin Waxes

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Chemical processes with hydrocarbons of various boiling ranges (gasoline, oils, waxes) frequently require knowledge of their contents of n-paraffins and isoparaffins, which has required tedious or expensive experiments; an easily workable chemical method for providing this information fills a definite need in hydrocarbon analysis. The method described in this paper is based on a simple chemical reaction with antimony pentachloride, followed by an indirect determination of the n-paraffins which have not been affected by this reagent, while the isoparaffins are converted into insoluble tarry matter. An accuracy of about 3 to 6% of total sample may be reached. The method applies also to other substances such as olefins, alcohols, fatty acids, etc., after they have been converted to hydrocarbons by usual methods. There are many possibilities of applying this simple and cheap analytical method in scientific and commercial hydrocarbon processes, as in most cases the contents of branched or normal hydrocarbon chains in the source material, in the intermediates, and in the final products reflects largely on the efficacy of the process and on the quality of the products. It is hoped, therefore, that petrochemistry will draw considerable advantages from this new method.

THE commercial value of hydrocarbon mixtures sometimes depends largely on their contents of straight carbon chains. In some cases, such as the preparation of detergents, unbranched hydrocarbons in the source material are preferred; on the other hand, *n*-paraffins in motor gasolines are harmful because of their low octane rating.

In spite of the considerable importance of this special analytical question, no simple chemical method of general applicability for the determination of straight-chain paraffins in hydrocarbon mixtures has been known, as there is but little difference in the chemical properties of *n*-paraffins and isoparaffins. This analytical field is chiefly covered by physical methods, such as the application of infrared absorption spectroscopy (*1*).

Some years ago Schaarschmidt and Marder (*3*) found a difference in the reaction rate of *n*-paraffins and isoparaffins with antimony pentachloride, and they based a rough and qualitative method for distinguishing *n*-paraffins from isoparaffins on this fact. Under the conditions outlined by these authors a quantitative distinction and separation of *n*-paraffins in hydrocarbon mixtures of various molecular weight are not possible. This

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Table I. Calculated Values of *n*-Paraffins

n-Paraffin	Boiling Point, ° C. 760 mm. Hg	15 mm. Hg	0.5 mm. Hg	d_{20}° C. (in CCl_4 Solution)
C_6H_{14}	69	...	...	0.650
C_7H_{16}	98	...	...	0.675
C_8H_{18}	126	...	...	0.695
C_9H_{20}	150	...	...	0.709
$C_{10}H_{22}$	173	...	...	0.720
$C_{11}H_{24}$	194	80	...	0.730
$C_{12}H_{26}$	215	93	40	0.740
$C_{13}H_{28}$	234	113	55	0.748
$C_{14}H_{30}$	253	128	70	0.755
$C_{15}H_{32}$	271	142	84	0.760
$C_{16}H_{34}$	288	155	96	0.764
$C_{17}H_{36}$	303	168	108	0.767
$C_{18}H_{38}$	317	180	118	0.770
$C_{19}H_{40}$	330	190	128	0.773
$C_{20}H_{42}$	345	203	140	0.775
$C_{22}H_{46}$	...	235	170	0.781
$C_{25}H_{52}$	...	252	190	0.785
$C_{30}H_{62}$	...	294	225	0.795

objective could be reached by modifying the reaction conditions and by applying a physical method for the determination of the *n*-paraffins which remain unchanged during this chemical reaction.

The reactivity of antimony pentachloride with hydrocarbons was decreased by applying carbon tetrachloride as a diluent, choosing concentration, reaction temperature, and time in such a way that isoparaffins react completely in the carbon tetrachloride solution, but *n*-paraffins remain practically unchanged.

As the separation of the unaffected *n*-paraffins from the carbon tetrachloride by distillation is impracticable in samples of lower boiling range, a physical method for the analysis of binary mixtures of *n*-paraffins and carbon tetrachloride based on the widely differing densities of both substances was outlined. The mixture is placed in a density bottle, and the *n*-paraffin content may be calculated from the density of the mixture by the mixture rule, using an adaptation of a pycnometric fat determination method (*2*).

APPARATUS

A 50-ml. bulb flask is attached to a Liebig condenser by a ground joint. A small calcium chloride tube is placed at the top of the condenser.

PROCEDURE

Weigh out such an amount of sample that about 0.7 to 1.0 gram of isoparaffins or naphthenes is present.

Add exactly 5 ml. of pure carbon tetrachloride with a pipet, dissolve completely, and add immediately 5 ml. of pure antimony pentachloride from a small graduated cylinder. Attach the condenser and cool by placing the flask in ice water for a short time, in case a vigorous reaction starts. Place the flask in a water bath at 40° C. and maintain at this temperature for 3 hours. If after 1 hour no reaction, indicated by a dark color and deposit of tarry matter, has taken place, add a small drop of vaseline oil, which contains no *n*-paraffins but may act as a starter, and heat 2 more hours at 40° C.

After 3 hours take the flask from the condenser, add exactly 25 ml. of carbon tetrachloride by a pipet, shake gently, and pour the carbon tetrachloride layer as completely as possible into a small separatory funnel, but avoid inclusion of the tarry matter.

Shake the carbon tetrachloride solution with 100 ml. of a mixture of 2 volumes of concentrated hydrochloric acid and 1 volume of water vigorously for about 1 to 2 minutes.

Place a small pledget of cotton in the dry stem of the separatory funnel and run the lower layer, which now contains only carbon tetrachloride and the unchanged hydrocarbons, into a 25-ml. density bottle. Place the density bottle in a water bath at exactly 20° ± 0.2° C. for half an hour, adjust to the mark, and weigh.

NOTES

It is necessary to keep these conditions of concentration, time, and temperature, which have been proved by many tests, because some isoparaffins need an excess of antimony pentachloride, whereas, *n*-paraffins of lower molecular weight may be affected by too great an excess of this reagent. If nothing is known about the approximate *n*-paraffin content of the sample, 1.5 to 2 grams are weighed out and the determination is repeated with the proper amount of the sample according to the result of the first determination.

A convenient and sufficiently accurate form of density bottle is a 25-ml. volumetric flask sealed with a glass stopper, and about 4 mm. in diameter at the neck. These flasks are easily filled and emptied by introducing a glass capillary tube in order to discharge the air.

CALCULATION

The *n*-paraffin content of the sample is calculated by the rule of mixtures from the density of the carbon tetrachloride solution obtained by the procedure described above

$$d\ (\text{mixture}) = \frac{\text{grams of } CCl_4 + \text{grams of paraffin}}{\text{vol. of } CCl_4 + \text{vol. of paraffin}} \qquad (1)$$

$$\%\ n\text{-paraffins} = \frac{30\ (d_{CCl_4} - d_{\text{solution}})}{\frac{d_{\text{solution}}}{d_{\text{paraff.}}} - 1} \times \frac{100}{\text{grams of sample weight}} \qquad (2)$$

$d_{\text{paraff.}}$ equals the density of the *n*-paraffin of the sample in carbon tetrachloride solution. This value has been shown to be about 1% lower than the density of the pure hydrocarbons without a solvent. The values of the *n*-paraffins calculated from the density of their solutions in carbon tetrachloride are listed in Table I. The value to be used for the calculation of the *n*-paraffin content by Formula 2 as $d_{\text{paraff.}}$ must correspond with the mean boiling point of the sample.

The volume of carbon tetrachloride has been fixed as 30 ml.

Table II. Tests

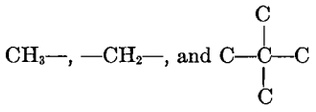
Substance	Sample, G.	% *n*-Paraffins Calcd.	Found	Diff.
Pure *n*-paraffins				
n-Heptane	1	100	100	0
n-Heptane	2.5	100	100	0
n-Heptane	4	100	99	−1
n-Nonane	2	100	100	0
n-Hexadecane	1	100	102	+2
n-Hexadecane	3	100	100	0
n-Eicosane	2	100	100	0
Cyclohexane	3	100	100	0
Pure isoparaffins, naphthenes, and olefins				
Hexadecene	0.7	0	2	+2
Decalin	0.8	0	2	+2
3-Methylheptane	0.8	0	3	+3
Vaseline oil	1	0	2	+2
Decalin + 3-methylheptane + iso-octane	0.6	0	3	+3
Mixtures				
n-Heptane + 3-methylheptane		11	10	−1
		26	22	−4
		50	43	−7
		85	80	−5
n-Heptane + Decalin		65	66	+1
n-Heptane + Decalin + iso-octane		49	53	+4
		65	66	+1
n-Heptane + Decalin + 3-methylheptane + iso-octane		25	25	0
		25	19	−6
		26	22	−4
		86	81	−5
n-Hexadecane + 3-methylheptane		59	59	0
		90	92	+2
n-Hexadecane + Decalin		11	17	+6
		56	58	+2
		89	90	+1
n-Hexadecane + hexadecene		58	57	−1
		14	18	+4
n-Eicosane + vaseline oil		12	12	0
		13	17	+4
		97	94	−3
		95	94	−1
n-Eicosane + synthetic isoparaffin		28	28	0
		52	56	+4
		59	63	+4
		93	97	+4
		96	98	+2

Pipets adjusted with water have to be adjusted to the actual delivery of 25 and 5 ml. of carbon tetrachloride.

DISCUSSION

This procedure is applicable to gasolines (mixtures of paraffins and naphthenes) from about six carbon atoms in the molecule, to oils and paraffin waxes if they are soluble in carbon tetrachloride at 20° C.

The following hydrocarbons are stable against antimony pentachloride: all saturated hydrocarbons containing only



groups, such as *n*-paraffins, cyclohexane, and compounds with quaternary carbon atoms such as 2,2-dimethylbutane. Thus, the true *n*-paraffin content of a hydrocarbon mixture is found by this method only if the other hydrocarbons listed in this group are absent; otherwise, their percentage is included in the *n*-paraffin content.

The following hydrocarbons react with antimony pentachloride to form products which are insoluble in carbon tetrachloride: compounds with a tertiary carbon atom such as isoparaffins, substituted cycloparaffins (naphthenes), and olefins.

Aromatic compounds and oxygenated compounds react with antimony pentachloride to form products which are partially soluble in carbon tetrachloride; thus they interfere with this method if present in considerable amounts, and must be removed before antimony pentachloride is applied, by treatment with sulfuric acid according to well-known procedures.

This method is not restricted to mixtures of paraffins and naphthenes, but applies also to olefins, alcohols, ketones, aldehydes, fatty acids, etc., after these compounds have been transformed into paraffins by adequate hydrogenation.

The accuracy of this method varies with the molecular weight of the sample; in oils and paraffin waxes the average absolute error is about 2 to 4% of the total sample and in gasolines the error is sometimes greater (3 to 6%), as may be learned from the data in Table II. It is probable that by a further study of the individual reaction rates of hydrocarbons with antimony pentachloride and by slight modifications of the procedure this range of errors may be narrowed.

ACKNOWLEDGMENT

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Infrared Analysis of Five C_{10} Aromatics

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The need for a means of analyzing mixtures of five C_{10} aromatics led to the method presented, which was designed to be rapid and accurate, and to have maximum simplicity of execution. No dilution of standards or of samples was required in either calibration or analysis. Measurements of cell length and absorbancy were eliminated by the condition of normalization and the use of reference wave lengths, respectively. The accuracy is shown to compare favorably with similar reported work, the error being 0.5% absolute over the whole percentage range. An infrared normalized multicomponent analysis of hydrocarbon liquids can be set up under the following restrictions, each of which materially simplifies performance of the analysis: assumption of validity of Beer's law, no dilution of standards or samples, and no knowledge of the length or absorbancy of the rock salt cell.

INTEREST in the composition of isomeric butyl and diethylbenzene mixtures boiling between 165° and 190° C. led to the development of a method for determining the composition of such mixtures. The analysis was set up using the ultraviolet region, but was found inadequate for complete determination of the individual isomers (*7*). A mass spectrometric determination (*5*) of traces of the C_{10} isomers in ethylbenzene has been reported, but determination of concentrations of the individual isomers was not indicated. No analysis of these mixtures by Raman spectrometry has been reported, although it has been indicated as a possible approach (*4*). Fractionation is not a sufficiently powerful tool to permit easy separation of the isomers, and no rapid and accurate chemical methods have been found in the literature. Resort was therefore made to infrared spectrophotometry; about 45 minutes are required to obtain results having an average absolute error of 0.5%.

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APPARATUS

A Perkin-Elmer Model 12C spectrometer equipped with a Model 51 breaker-amplifier and Brown recorder was used.

HYDROCARBONS

Sources and given purities of the hydrocarbons used were as follows:

Compound	Purity, Mole %	Source
o-Diethylbenzene	99.95 ± 0.03	National Bureau of Standards
m-Diethylbenzene	99.93 ± 0.04	National Bureau of Standards
p-Diethylbenzene	99.93 ± 0.02	National Bureau of Standards
Isobutylbenzene	99.87 ± 0.09	National Bureau of Standards
sec-Butylbenzene	99 minimum	Phillips Petroleum Co.

DEFINITIONS

The following terms and meanings are used in this paper:

$$A = \log \frac{I_0}{I} = Kcd$$

Table I. Wave Lengths, Slit Widths, and Shutters Used

Substance	Analytical λ, μ	Analytical Slit width, mm.	Analytical Shutter	Reference λ, μ	Reference Slit width, mm.	Reference Shutter
o-Dimethylbenzene	10.65	0.375	LiF	10.53	0.368	LiF
m-Diethylbenzene	11.54	0.450	LiF	11.40	0.440	LiF
p-Diethylbenzene	8.92	0.262	Glass	8.79	0.255	Glass
sec-Butylbenzene	10.00	0.330	LiF	10.53	0.368	LiF
Isobutylbenzene	8.56	0.255	Glass	8.79	0.255	Glass
sec-Butylbenzene (to track monochromator)				9.78	0.130	

where

A = absorbancy (analytical absorbancy indicates an absorbancy at an analytical wave length; reference absorbancy, an absorbancy at a reference wave length) (*8*, *10*)
K = absorbancy coefficient
c = concentration
d = cell length
I_0 = intensity of incident radiation
I = intensity of transmitted radiation

$$\text{Absolute error} = (\%\ \text{known}) - (\%\ \text{found})$$

$$\text{Relative error} = \frac{(\%\ \text{known}) - (\%\ \text{found})}{(\%\ \text{known})}$$

PROCEDURE

Selection of Absorbance Bands, Slit Widths, and Shutters. To secure good intercomparison of spectral position and band absorbancy, spectra of all hydrocarbons to be included in the analysis were superimposed on the same chart. Because the analysis was intended to apply equally well to all percentage ranges, absorbance bands were selected which have about equal absorbancy. Of secondary importance was the fact that the bands show only slight mutual interference, although this is desirable.

Careful scanning of the pure hydrocarbons established the wave lengths of the selected analytical bands to better than 0.004 micron. The 9.78-micron band of *sec*-butylbenzene was used as a means of subsequently tracking the wave-length shift of the monochromator with temperature. Three wave lengths, characterized by low absorbancy for all components and by relative nearness to the analytical wave lengths, were also selected to furnish reference absorbancies to be subtracted from relevant nearby analytical absorbancies, thereby accurately canceling out the absorbance of the cell.

Slit widths were used which would furnish at the selected wave lengths thermocouple signals of approximately 3 microvolts, which were sufficient to eliminate error from noise with the instrument at hand. This was considered preferable to retention of smaller slit widths.

To attempt to reduce errors from stray light, a glass shutter was used for all wave lengths longer than 6.2 microns, and a lithium fluoride shutter at positions beyond 9.2 microns.

The combinations of analytical and reference wave lengths, slit widths, and shutters used are shown in Table I.

Method of Obtaining Absorbancy. The following method was devised for the Perkin-Elmer system which utilizes a chart ruled for log $1/N$, incorporates means for putting test signals into the amplifier, and uses the Brown recorder. The method must be altered if necessary for use on systems having different characteristics.

A test signal of 0.1 microvolt was used in conjunction with all readings for absorbancy. Introduction and subsequent removal of the signal immediately before each reading tended to ensure that all readings (or settings of the pen) were taken on the same side of true balance, so that errors from recorder dead space were minimized.

The ruling of the scale used—log $1/N$—implies that the full-scale signal should be identically that of I_0, the incident radiation. Achieving this identity ordinarily requires time-consuming manipulation of the interacting gain and balance controls. However, this identity is not necessary; it is only necessary that the pen fall accurately on infinity (log 1/zero), and this can be achieved with only a rather coarse adjustment of the balance control. With the shutter in the beam, and after deflection of the pen by the test signal as has been indicated, the ruling for infinite absorbancy is placed directly under the pen by a manual lateral shifting of the metal scale. Placement of the pen within the 0.125-inch (0.6-cm.) lateral range available to the metal scale can be easily accomplished.

The deflection for I_0 was made to fall somewhat short of full scale, and the reading from the log $1/N$ scale was subtracted from the corresponding reading for I:

$$\log 1/I - \log 1/I_0 = \log I_0/I$$

By means of these techniques, the eight absorbancies used in this analysis could be accurately obtained in about 20 minutes.

Absorbancy Coefficients. Absorbancy coefficients of the diethylbenzene isomers, *sec*-butylbenzene, and isobutylbenzene were obtained by measuring absorbancies of these compounds without dilution by solvents. Wave-length settings were always approached from shorter wave lengths, and slit widths approached from smaller slit widths. Occasional negative figures were produced in the subtraction of the reference absorbancies, and these were handled identically in the calculations, with preservation of sign. The relative values of the coefficients are given in Table II.

ANALYSIS

Samples were also measured without dilution, by the same procedure in which the absorbancy coefficients were obtained.

The method of Crout (*2*) was used in calculations. Results for one sample could be calculated in 10 to 15 minutes.

All results were normalized; this not only canceled out changes in cell length but allowed cells of any length to be used so long as prohibitively high (roughly, over 1.1) absorbancies did not occur. Analysis of synthetics and independence of results from cell length are shown in Table III. Because the cell length need not be known during calibration or analysis, it should be chosen to have the maximum permissible length, so as to improve determination of the smaller coefficients and absorbancies. The cell length should not change while absorbancy coefficients are being obtained. Although this constancy was not checked by actual measurement, it is strongly implied by the analytical results in Table III. Precision and accuracy of the method are shown in Table IV.

Table II. Relative Values of Absorbancy Coefficients

Wave-Length Combinations for Absorbancy Figures, μ	Isobutylbenzene	*sec*-Butylbenzene	*o*-Diethylbenzene	*m*-Diethylbenzene	*p*-Diethylbenzene
8.56–8.79	0.870	0.031	0.002	0.215	−0.063
10.00–10.53	−0.068	0.529	−0.083	0.053	0.003
10.65–10.53	−0.074	−0.142	0.395	−0.021	−0.013
11.54–11.40	−0.079	0.004	0.056	0.591	0.064
8.92–8.79	0.244	0.023	0.071	0.020	0.760

Table III. Analyses of Synthetics, Weight Per Cent

	K	F[a]	F[b]	K	F[a]	K	F[a]	K	F[a]	K	F[a]	F[b]
Isobutylbenzene	22.2	22.1	21.8	47.0	46.1	0.0	0.0	21.5	21.2	13.5	14.2	13.7
sec-Butylbenzene	23.6	23.3	23.5	31.8	31.4	33.8	33.2	15.5	16.0	17.6	17.7	18.0
o-Diethylbenzene	22.4	22.8	22.8	0.0	0.9	36.6	36.2	7.5	8.1	9.1	9.9	9.8
m-Diethylbenzene	11.2	11.4	11.6	0.0	1.0	11.3	11.2	16.6	16.2	18.3	17.5	18.7
p-Diethylbenzene	20.5	19.8	20.2	21.2	20.0	18.3	19.3	38.9	38.5	41.4	40.7	40.0

K. Known.
F. Found.
[a] 0.100-mm. cell used.
[b] 0.203-mm. cell used.

Table IV. Survey of Comparable Analyses

Reference	Average No. Components	Average Absolute Error	Average Relative Error
Present method	5	0.5	2.7
(9)	2.6	0.5	1.8
(3)	3.6	0.9	4.3
(1)[a]	4	0.4	1.5
(6)	4	1.0	4.2

[a] Ten-component analysis not included (average absolute error, 0.7%; average relative error, 16.9%).

COMPARISON OF RESULTS

The recent literature was consulted for analyses of liquid multicomponent solutions, in order to obtain a means of judging the precision and accuracy of the present analysis. The survey is not claimed to be complete, but the results are probably representative at this time of this type of work—namely, infrared multicomponent analysis of liquids where all components sought total 100% and are generally present in concentrations greater than 3 to 5%, and in which high-absorbancy techniques are not used. Results are shown in Table IV.

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Colorimetric Determination of Rhenium

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A method is described for the quantitative colorimetric determination of rhenium in samples containing as little as 0.1 microgram to 2.5 mg. of rhenium per gram of sample. Determinations of rhenium have been made in the presence of 125 mg. of molybdenum in a volume of 25 ml. The molybdenum is separated from the rhenium as a metalorganic compound, formed with ethyl xanthate, and extracted from a dilute acid solution with an organic solvent mixture. The rhenium is determined by forming the rhenium thiocyanate color complex and measuring the transmittance of the solution with an electrophotometer. A precision of ±2% has been obtained over the specified concentration ranges. The method is readily applicable to the mass analysis techniques of routine analytical laboratories, and permits a rapid and accurate search for rhenium in minerals.

IT BECAME desirable to work out a method for the quantitative determination of small amounts of rhenium in the presence of large amounts of molybdenum. A method that would be faster, and if possible more accurate than existing methods, was sought. A procedure has been developed which not only permits a quantitative rhenium determination to be made much more rapidly, but also lends itself to a mass analysis technique.

The new method differs from the older distillation techniques in that the distillation of rhenium from an acid solution with its attendant difficulties is eliminated. The molybdenum, a common interferring ion in ores and solutions containing rhenium, is separated from the rhenium as a metalorganic compound, formed with ethyl xanthate, and extracted from a dilute acid solution with an organic solvent mixture.

Two other methods of rhenium analysis were previously used. The method of Hiskey and Meloche (*3*) was modified by making an ether extraction to concentrate the rhenium color for easier color comparisons. A modified Hoffman and Lundell (*4*) method was developed at the Chase Brass Laboratory (*7*). A colorimetric method for determining rhenium, limited to samples containing no more than 1 mg. of molybdenum, which was precipitated with α-benzoinoxime, was developed by Melaven and Whetsel (*6*). Geilmann and Bode (*1*) did considerable work on the colorimetric method for the determination of rhenium by forming the rhenium thiocyanate color complex.

As various metallic salts precipitate molybdate ions and not perrhenate ions, separations of molybdenum and rhenium based on the insolubility of calcium molybdate, barium molybdate, and lead molybdate were tried, but later abandoned in favor of the xanthate method because of its greater efficiency. The use of xanthate for the separation of molybdenum had been worked out previously in this laboratory in some detail by Hansen (*2*) and Hurd (*5*).

REAGENTS REQUIRED

Sodium Ethyl Xanthate Solution. Dissolve 40 grams of xanthate in 60 ml. of distilled water, filter, and dilute the filtrate to 100 ml. The water solution of xanthate should be prepared fresh daily.

Sodium Thiocyanate Solution. Dissolve 200 grams of C.P. sodium thiocyanate in distilled water and dilute to 1 liter with distilled water.

Stannous Chloride Solution. Dissolve 350 grams of C.P. stannous chloride in 250 ml. of concentrated hydrochloric acid at room temperature with occasional stirring. Add 250 ml. of distilled water and dilute to 1 liter with 1 to 1 hydrochloric acid. Add a few pieces of tin to keep the solution reduced.

Ether for Dilution. Place 25 ml. of 1 to 2 hydrochloric acid, 2 ml. of the sodium thiocyanate solution, and 2 ml. of the stannous chloride solution in a 125-ml. glass-stoppered separatory funnel, and add 30 ml. of purified ethyl ether. Shake for 30 seconds and allow the two resulting layers to separate. Drain off the acid layer and reserve the ether layer.

Standard Rhenium Solution. Dissolve 0.0775 gram of pure potassium perrhenate in distilled water, add 25 ml. of 6 *N* sulfuric acid, and dilute to 500 ml. with distilled water. This gives a solution containing 0.10 mg. of rhenium per ml. An 0.01-mg. rhenium solution may be prepared by a tenfold dilution with 0.3 *N* sulfuric acid.

PROCEDURE

Preparation of Standard Curve. Use a solution containing 0.01 mg. of rhenium per ml. Pipet into 250-ml. beakers 1-, 2-, 5-, and

10-ml. aliquots, in duplicate, add water to bring volume to 25 ml., add 2 or 3 drops of concentrated hydrochloric acid and 15 ml. of saturated bromine water, and 4 or 5 drops of liquid bromine, and then proceed exactly as outlined in the analytical procedure after the addition of bromine.

Prepare the standard curve by plotting the values obtained against the milligrams of rhenium.

Procedure for Samples Containing More than 5% Molybdenite. Fuse cautiously 1.250 grams of the sample in a 50-ml. Armco iron crucible with 5 grams of sodium hydroxide over a gas burner. After effervescence has ceased, add 5 grams of fresh sodium peroxide and fuse strongly over a hot gas flame until the sample is completely decomposed and appears homogeneous.

After fusion, pour the melt onto a clean iron slab (a piece of boiler plate 0.75 inch thick and 6 to 8 inches, 15 to 20 cm., square) and allow it to harden. Transfer the empty crucible to a 250-ml. beaker and cover. Transfer the melt to a 400-ml. beaker and cover. After cooling, fill the crucible with water and heat for a few minutes, then rinse thoroughly and scrub with a rubber policeman. Pour the washings and solutions from the crucible cautiously and slowly into the beaker containing the melt, with the cover lid pulled back just far enough to allow the liquid to be poured into the 400-ml. beaker. The total volume should be about 150 ml. The solution will be brown with a heavy suspension of iron hydroxide. Heat the solution to boiling, add a little macerated filter paper pulp, and filter at once through a Whatman No. 3 filter paper, using a Büchner funnel and suction. Wash five times with hot water. Chill the solution in an ice bath. A small iron precipitate will usually form. Filter and wash again. Transfer to a 250-ml. volumetric flask, dilute to the mark with distilled water, and mix well.

Table I. Determination of Known Amounts of Rhenium in Presence of Molybdenum

Sample,	Molybdenum Present *Mg.*	Rhenium Present *Mg.*	Rhenium Found *Mg.*	Error %
1	100.0	0.100	0.096	4.0
2	100.0	0.100	0.096	4.0
3	100.0	0.100	0.094	6.0
4	100.0	0.100	0.100	0.0
5	100.0	0.100	0.100	0.0
6	100.0	0.030	0.030	0.0
7	100.0	0.030	0.030	0.0
8	100.0	0.030	0.029	3.0
9	100.0	0.030	0.029	3.0

Pipet 25-ml. aliquots of the above solution into 250-ml. beakers. Neutralize the sample with concentrated hydrochloric acid and add 3 drops in excess. Add 10 drops of liquid bromine. Place the sample on a hot plate at low heat and heat without boiling, until the excess bromine is driven off. Add 5 *N* sodium hydroxide solution until the sample is neutralized, and 2 or 3 drops in excess until a pH of 9 to 11 is reached. Transfer to a glass-stoppered separatory funnel (250-ml.) and cool in running water. When the sample has reached room temperature, add 5 ml. of the xanthate solution and mix, add 8 ml. of concentrated hydrochloric acid, shake for 5 seconds, and finally add 50 ml. of 1 to 1 mixture of carbon tetrachloride and benzene. Shake vigorously for 30 seconds, let stand until separation is complete, and swirl to collect all the solvent at the bottom. The water layer should be colorless or pale pink. The solvent layer will be an intense red-violet color. Draw off the solvent layer and discard it. Add 25 ml. of the solvent mixture, washing off stopper and lip of funnel, and shake for 15 seconds; again allow the layers to separate. Draw off the solvent layer. Repeat the extraction with another 25 ml. of solvent. If the solvent layer is not colorless after the third extraction, make one more extraction.

To the sample solution in the separatory funnel add 10 drops of liquid bromine and shake vigorously, allow to stand 10 minutes, add 25 ml. of solvent mixture, shake 20 seconds, and allow the phases to separate. Draw off and discard the solvent mixture. The volume should be approximately 80 ml.; add 40 ml. of concentrated hydrochloric acid or one half the volume of the solution.

Transfer back to the separatory funnel and cool. Add 5 ml. of the sodium thiocyanate solution and 5 ml. of the stannous chloride solution. Shake the sample for 10 seconds and allow to stand 30 minutes.

After standing, add 20 ml. of purified ethyl ether and shake for 20 seconds, allow the phases to separate, and draw the aqueous layer into a second separatory funnel. Drain the ether layer into a 50-ml. volumetric flask. Rinse the first separatory funnel with 15 ml. of ethyl ether and drain into the second separatory funnel. Add 1 ml. of stannous chloride solution and shake for 15 seconds. Allow the phases to separate and drain the aqueous layer into the first separatory funnel.

Add the ether layer to the 50-ml. volumetric flask. Repeat the extraction with 15 ml. more of ether, adding another milliliter of stannous chloride solution. After separation discard the aqueous layer, add the ether layer to the 50-ml. volumetric flask until it is filled to the calibration mark, and discard the balance, or add "ether for dilution" as necessity dictates.

Table II. Comparison of Rhenium Determinations

Sample	A. Rhenium Found, G./G. of Sample	B. Rhenium Found, G./G. of Sample 1st fusion	2nd fusion	3rd fusion
1	0.00030	0.00031	0.00029	0.00029
	0.00030	0.00030	0.00030	0.00030
	0.00024	0.00031		
2	0.00031	0.00035	0.00033	0.00031
	0.00030	0.00035	0.00035	0.00031
	0.00032	0.00034		
3	0.00026	0.00031	0.00031	0.00032
	0.00029	0.00031	0.00031	0.00032
	0.00030	0.00032		
4	0.00028	0.00031	0.00031	0.00033
	0.00025	0.00031	0.00032	0.00032
	0.00027	0.00031		
5	0.00028	0.00028	0.00032	0.00030
	0.00027	0.00028	0.00031	0.00030
	0.00028	0.00030		
6	0.00028	0.00032	0.00032	0.00031
	0.00026	0.00032	0.00031	0.00028
	0.00028	0.00032		

A. Distillation of rhenium from 250-mg. samples of molybdenite.
B. Removal of molybdenum by xanthate method, fusions of 1.250 g. of molybdenite prepared to 250-ml. volume, 25-ml. aliquots used.

Transfer a portion to an absorption cell of an electrophotometer and measure the transmittancy or absorbancy at approximately 420 mu.

A blank sample may be prepared to set the zero point on the photometer, but because it checks the ether for dilution, prepared ether may be used to set the instrument to zero.

Using the value obtained, read from the standard curve the number of milligrams of rhenium present.

Calculate the percentage of rhenium present.

Procedure for Samples Containing Less than 5% Molybdenite. Add sufficient nitric acid to cover a sample which may vary in weight from 10 to 100 grams, depending on the concentration of rhenium present, and digest cautiously with the nitric acid without boiling. (Extreme caution is used if the sample is high in sulfides or carbonates.) Samples should contain between 0.02 and 0.15% mg. of rhenium. After the initial reaction is completed add 25 ml. of hydrochloric acid and complete the digestion. Filter the sample and wash thoroughly.

Place the filtrate and washings on the hot plate and evaporate down without boiling. Add hydrochloric acid repeatedly in 25-ml. increments until all the nitrates are removed. Usually six additions are required.

After all the nitrates have been removed from the sample, add 200 ml. of distilled water. Under continuous stirring, dust in cautiously sodium peroxide, until the sample is basic. Filter and wash immediately. Add filtrate and washings to a 500-ml. separatory funnel, add xanthate solution, and proceed as for samples containing more than 5% molybdenite.

DISCUSSION

Fusions. Fusions have been successfully made on samples from 0.25 to 2.5 grams. However, fusions made on 1.250 grams were easily handled, and better volume control was gained by fusing the larger sample and taking aliquot parts after filtration.

More consistent results were obtained by boiling and filtering. then chilling the filtrate and refiltering before diluting to volume, The method was designed for samples containing approximately 0.03% rhenium. Larger or smaller aliquots can be taken, depending on the amount of rhenium present.

If sample solutions contain a blue tinge after filtration, reduced molybdenum compounds will be found. The melt should be kept away from the iron crucible after solution with water, to avoid the reducing action of the iron.

Fusions have been made using sodium hydroxide alone, but the fusions were very difficult to dissolve and the molybdenum was strongly reduced. Answers were always erratic. Varying amounts of sodium peroxide were used, but it seemed necessary to have four times the sample weight of sodium peroxide present; these fusions dissolve very readily and completely.

Bromine. Whenever the sample solution had a blue tinge of reduced molybdenum after the first solvent extraction, results were highly erratic. Several oxidants were employed, but bromine when used as outlined in the procedure eliminated the blue color and the erratic results. Hydrogen peroxide (30%) also gave excellent results, but was slower and a large excess was needed.

pH. Erratic results were obtained on samples whose pH was less than 9 prior to the addition of the sodium ethyl xanthate. Consistent results were obtained when the solutions were adjusted to a pH range of 9.0 to 11.0.

Xanthate. Purified sodium ethyl xanthate in a filtered aqueous solution is more effective than a dry powder. A large excess of xanthate is needed to remove all the molybdenum from the sample solution, provided the molybdenum is in the highest state of oxidation. Sodium ethyl xanthate will not extract reduced molybdenum. If reduced molybdenum is present, erratic high results will be obtained. A blue tinge after the xanthate extractions indicates the presence of reduced molybdenum.

Solvents. It was found that most water-insoluble organic solvents would extract the molybdenum xanthate complex. Benzene was used at first, but it was determined that a 1 to 1 mixture of carbon tetrachloride and benzene was superior to benzene alone, as the combination permitted the bottom draining of the material to be discarded, and thereby minimized the loss of the rhenium-containing solution. The mixture was found superior to carbon tetrachloride alone, as a more complete removal of the molybdenum was effected. Chloroform as recommended by Hurd (*5*) was tried, but not used, because of its higher volatility.

Stannous chloride. Erratic results were traced to a slight instability of the final rhenium complex color. This seems to be eliminated by addition of 1 ml. of stannous chloride solution after each ether extraction of the final sample solution.

All color measurements were made in a Fisher's A. C. electrophotometer, using the microcells as supplied with that instrument, and their standard blue filter having a maximum transmittancy of 425 mu.

Complete analyses have been made in less than 3 hours with this method. On a series of molybdenite samples, the rhenium determination increased in value equal to added increments of potassium perrhenate.

The only interference studies made have been those where sodium selenite has been added to samples and standards. The selenium is removed along with the molybdenum in the xanthate separation and has given no trouble. Copper and iron are removed by the filtration after fusion.

Work carried out by Hoffman and Lundell (*4*) shows that the following elements will be noninterfering in this procedure: cerium, cobalt, chromium, gallium, germanium, indium, iridium, lead, nickel, osmium, ruthenium, thallium, uranium, and vanadium (all in 2-mg. quantities). Chromium in large amounts (40 mg.) imparts a slightly green tint to the ether layer. Platinum, rhodium, and tungsten interfere by coloring the ether layer.

Geilmann and Bode (*1*) have carried out a very comprehensive work on the rhenium thiocyanate complex. They point out that any peroxides present in the ether will give low results, that the presence of ferric chloride will give erratic results, and that all nitrates must be avoided, for in the presence of potassium thiocyanate and stannous chloride, a yellow coloration is developed which may be extracted by the ether. The same amount of neutral salts must be present in the solutions by which the standard curve is prepared, as will be expected in the unknown samples.

RESULTS

Following the above procedures, the transmittance was determined on a series of samples containing 0.1 microgram to 2.5 mg. of rhenium. Aliquot portions were taken of samples high in rhenium, such that a 50-ml. ether extraction did not exceed 0.2 mg. of rhenium, thereby keeping the transmittance of the rhenium complex well within the range of Beer's law. These samples ranged as high as 125 mg. in molybdenum. It was possible to determine rhenium on samples containing 250 mg. of molybdenum but this amount of molybdenum necessitated a double extraction of the sample, with a corresponding loss in rhenium.

In Table I the results are given of synthetic samples prepared by using molybdic oxide and potassium perrhenate.

In Table II the comparative results are given of various samples, high in molybdenum, which were determined by two methods: separation of the rhenium from the molybdenum by distillation, and separation of the rhenium from the molybdenum by the use of sodium ethyl xanthate.

In Table III the results are given of rhenium determinations made on samples that contained microgram quantities of rhenium.

Table III. Rhenium Determinations by Analytical Procedure for Samples Containing Less than 5% Molybdenite

100-G. Sample	Rhenium Found, Mg.
1	0.036
	0.035
	0.036
2	0.032
	0.031
	0.031
3	0.019
	0.018
	0.017
4	0.13
	0.13
	0.13

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Colorimetric Determination of Nickel with Alpha-Furildioxime

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The work was undertaken to study the conditions under which nickel may be determined colorimetrically by means of α-furildioxime, especially as compared with dimethylglyoxime or 1,2-cyclohexanedionedioxime. The nickel–α-furildioxime complex is a usable form for determining nickel, following extraction of the colored complex with 1,2-dichlorobenzene. This extractability facilitates separation of the metal from colored interfering solutions, such as iron or chromate salts. The pH of the solution must be regulated, but otherwise the system has satisfactory colorimetric properties. The general result is a method having some advantage over the earlier dioxime processes. Its applicability was shown by the determination of nickel in a magnesium alloy and in National Bureau of Standards steel No. 13c.

NEARLY a decade ago (*4*) di(2-furyl)ethanedionedioxime (α-furildioxime) was recommended for the colorimetric determination of nickel in magnesium alloys. Subsequently, the procedure was adopted by the American Society for Testing Materials (*2*). Previously this compound had been investigated for use as a reagent for spot tests (*3*) and as a precipitant for the gravimetric determination of nickel (*7*, *10*). A critical study of the colorimetric method was started by Mitchell (*6*) following the observation of certain anomalies in the extraction operation.

The method involves the extraction of the stable nickel(II) complex with an immiscible organic solvent. The nickel is thus conveniently separated from many constituents in the sample, accompanied by the simultaneous formation of a yellow color suitable for colorimetric measurement.

APPARATUS AND REAGENTS

Transmittancy measurements were made in 1.000-cm. cells with either a General Electric recording spectrophotometer set at a spectral band width of 10 mμ, or with a Beckman D.U. spectrophotometer operating close to 1 mμ band width.

The stock solution of nickel sulfate was prepared by suitable dilution of a solution analyzed electrolytically for nickel.

The α-furildioxime, prepared by the method of Reed, Banks, and Diehl (*8*), had a melting point of 171–171.5° C. Solutions of the reagent were prepared in 95% ethyl alcohol. Redistilled technical (95%, Eastman) 1,2-dichlorobenzene was used in the extractions. Before use, the organic solvent was shaken with a solution of sodium acetate to remove traces of acidic substances that might be present. Solutions of chlorides, nitrates, or sulfates were used in the study of the effect of cations upon the extraction process and the color development. Alkali salts were used to study the effect of various anions.

Separatory funnels (125-ml. Squibb type) were most convenient for the extractions.

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

COLOR REACTION

Effect of Solvent. Nickel(II) forms a complex with α-furildioxime which is insoluble in water and many water–organic solvent combinations. In a slightly alkaline aqueous solution a yellow hue develops which rapidly fades as the complex precipitates. The solubility and stability of both the nickel(II) and the oxidized nickel complexes were tested in various solvents. The following solvents with water (1 to 1) did not form suitable colored systems for nickel(II) α-furildioxime: acetone, dioxane, ethyl alcohol, ethyl Carbitol, ethyl Cellosolve, isopropyl alcohol, methanol, and methyl Carbitol. Pyridine forms a yellow solution. Chloroform, 1,2-dichlorobenzene, diethyl ether, and ethyl acetate extract the complex from an aqueous solution. Carbon tetrachloride, *n*-amyl alcohol, nitromethane, nitropropane, tributylamine, and trichloroethylene do not extract the complex.

The complex in the presence of ammonium hypobromite appears to be stable only in isopropyl alcohol and pyridine solutions. Oxidation in an alkaline potassium persulfate solution caused a hue to develop similar to that of the dimethylglyoxime complex, but the color faded rapidly.

1,2-Dichlorobenzene was considered to be the best solvent tested because of its low volatility, nonflammable nature, immiscibility with water, and density (greater than that of water and of adequate difference for rapid separation of the layers). Extractions with this solvent required the presence of a small amount of ethyl alcohol to prevent turbidity in the organic phase.

The nickel(II) complex is unique for its color because nickel dimethylglyoxime and nickel 1,2-cyclohexanedionedioxime form almost colorless solutions in chloroform or 1,2-dichlorobenzene. Nickel(II) 1,2-cycloheptanedionedioxime is similar to the α-furildioxime complex in that it is very soluble in chloroform, but it is not as highly colored. One milligram of nickel α-furildioxime can be readily extracted with 1,2-dichlorobenzene or chloroform, whereas the dimethylglyoxime (*5*) and 1,2-cyclohexanedionedioxime complexes are not appreciably soluble in these solvents. The extractability of these other complexes will be discussed in another paper.

Effect of pH. The pH range for optimum color development occurs from pH 7.5 to 8.3. Below or above this range extraction is slow and incomplete. This narrow range necessitates the use of a buffer. Because the 1,2-dichlorobenzene was found to contain acidic constituents, the solvent is washed with a slightly basic solution prior to use. The effect of pH upon the extraction of nickel α-furildioxime is shown in Table I.

Table I. Effect of pH upon Extraction of Nickel(II) α-Furildioxime with 1,2-Dichlorobenzene

(0.1 mg. of nickel)

pH	Recovery, %
6.5	72
7.0	99
7.5	100
7.7	100
8.1	100
8.3	100
8.5	99
9.1	78
9.5	23

Volumes. Aqueous phase, 25 ml.; organic solvent, 5 ml. Number of successive extractions, 5.

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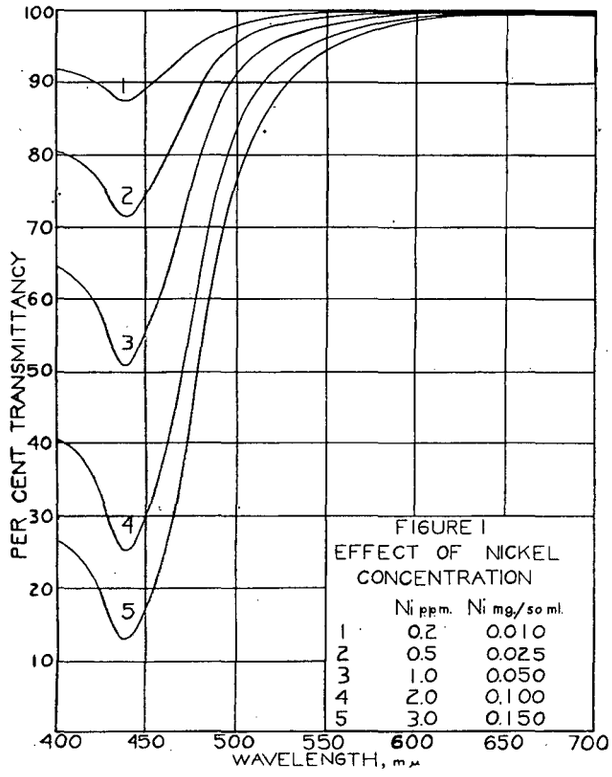

Effect of Ammonia and Ammonium Ion Concentration. Ammonia affects the extractability of the nickel(II) complex in much the same way as it does the precipitation of nickel. High concentrations of ammonia prevent color formation and extraction of the nickel. However, small concentrations of ammonium ion added as ammonium chloride up to 0.225 gram do not interfere with the extraction. Data in Table II show the effect of various concentrations of ammonium ion upon the color development and extraction process. Although a small amount of ammonia may be used to adjust the pH of the aqueous solution, it is recommended that sodium acetate or dilute sodium hydroxide be used instead.

Table II. Effect of Ammonium Ion upon Extraction of Nickel(II) α-Furildioxime with 1,2-Dichlorobenzene

(0.1 mg. of nickel)

Ammonium Ion, G.	Recovery, %
1.124	10
0.562	56
0.450	79
0.337	92
0.280	99
0.225	100
0.168	100
0.112	100
0.010	100

Volumes. Aqueous phase, 25 ml.; organic solvent, 5 ml. Number of successive extractions, 5.

Effect of Nickel Concentration. With a 1.000-cm. cell the transmittancy varies from 87.7% (0.2 p.p.m.) to 13.0% (3.0 p.p.m.) and the system conforms to Beer's law. The wave length of maximum absorption for the yellow solution occurs at 438 mμ (see Figure 1). The reference cell contained 1,2-dichlorobenzene, but water may be used.

Stability of the Hue. The transmittancy does not change over a period of 14 days in diffuse light at a temperature from 25° to 35° C. The transmittancy of a solution maintained at 100° C. in a water bath for 1 hour remained constant.

EXTRACTION PROCESS

Volume of Aqueous Phase. Satisfactory extractions are obtained when the volume of 1,2-dichlorobenzene is 5 ml. and the aqueous solution is between 20 and 75 ml. When the volume of the aqueous phase is greater than 75 ml., the extraction process is less efficient. An increase in the volume of the organic solvent to 10 ml. does not increase the extraction efficiency, as shown in Table III. The data were obtained by extracting successively five times with the volume of solvent indicated.

Table III. Effect of Volume of Aqueous Phase upon Extraction Efficiency

Volume of Aqueous Phase	Extraction Efficiency: 5 ml. of 1,2-dichlorobenzene	Extraction Efficiency: 10 ml. of 1,2-dichlorobenzene
Ml.	%	%
20	100	100
60	100	100
80	96	98
100	96	96
150	...	63

Number of Extractions. From two to three successive extractions with 5-ml. portions of 1,2-dichlorobenzene are sufficient to remove the nickel complex from the aqueous solution. Complete removal is readily detected by the absence of color in the organic solvent.

Reagent Concentration. A volume of 3.5 ml. of a 0.1% ethanolic solution of α-furildioxime is required to extract 0.1 mg. of nickel. As much as 15 ml. of reagent gave no adverse effect.

Order of Addition of Reagents. Addition of the reagent before the organic solvent did not impair the efficiency of the extraction, even if a slight precipitate of nickel α-furildioxime formed in the aqueous phase. However, less shaking is necessary if the mixture is shaken before the precipitate is allowed to form.

Effect of Electrolytes upon Extraction. The effect of the presence of various electrolytes at different concentrations upon the extraction was studied. No deleterious effect is experienced when extractions are carried out from solutions 0.05 to 2.5 *M* with respect to sodium acetate. Chloride ion up to 3.6 *M* does not affect the extraction. In these extractions 2 ml. of 3 *M* sodium acetate were added to make the solution pH 7.6 to 7.8. Nitrate below 2.0 *M* and sulfate below 0.63 *M* with 5 ml. of 3 *M* sodium acetate present (total volume of aqueous phase = 32 ml.) allow satisfactory results, but higher concentrations of both ions cause "salting-in" effects—i.e., the complex is not extracted completely. Addition of 5 ml. more of 3.0 *M* sodium acetate, or a total of 10 ml., eliminated the salting-in effect.

Extractions from almost saturated solutions of electrolytes are more difficult to carry out than with dilute solutions because the small difference in density between the aqueous and 1,2-dichlorobenzene layers causes the phase to separate more slowly. Dilution with water decreases the density of the aqueous phase, so that the phases separate more quickly. If it is not feasible to increase the volume of the aqueous phase one may use chloroform, which has a higher density than 1,2-dichlorobenzene.

Effect of Diverse Ions. In the study of the effect of diverse ions upon the color development and extraction process, 25 mg. of diverse ion were tested with 0.1 mg. of nickel(II). In all cases 5 ml. of 3 *M* sodium acetate (except in the test for acetate ion) and sufficient dilute hydrochloric acid or sodium hydroxide were added to adjust the solution to a pH of approximately 8.1. The pH was conveniently adjusted visually by addition of 2 drops of 0.5% ethanolic solution of phenolphthalein and then acid or base until the color due to the indicator was just perceptible.

The pH of colored solutions was adjusted by means of a pH meter. If 25 mg. of diverse ion did not interfere, it was assumed that the ion would not interfere under the usual conditions of determination, although this may not hold true if certain combinations of ions should be present.

The following ions do not interfere with the extraction of nickel α-furildioxime in concentrations as high as 25 mg. with 0.1 mg. of nickel(II) present in the aqueous phase of approximately 25 ml.: acetate, arsenate, arsenite, barium, benzoate, bromide, cadmium, calcium, chloride, chlorate, dichromate, fluoride, formate, hydrogen sulfite, iodate, iodide, lactate, lithium, magnesium, molybdate, nitrate, nitrite, oxalate, orthoborate, orthophosphate, persulfate, potassium, pyroborate, salicylate, selenate, sodium, strontium, sulfate, sulfite, tartrate, thiocyanate, thiosulfate, tungstate, and vanadate. Lead and uranyl ions form a very slight precipitate that does not interfere with the extraction.

The following ions, unless complexed, precipitate at the pH of the extraction: aluminum, antimony, beryllium, bismuth, cerium, chlorostannate, chlorostannite, chromium(III), cobalt, copper, iron(II), iron(III), manganese(II), mercury, thorium, titanium, zinc, and zirconium.

Permanganate interferes by reacting with the reagents. Gold, silver, platinum, and palladium form yellow solutions with the reagent. Perchlorate, pyrophosphate, cyanide, periodate, sulfide, and citrate prevent extraction of nickel. Silicate, in concentrations greater than 100 mg., slowly reacts with the ethyl alcohol to form a precipitate which interferes with the extraction. The extent of the error caused by the more important interferences is summarized in Table IV.

Table IV. Effect of Diverse Ions

Ion	Added as	Amount Mg.	Error[a] %	Amount Permissible Mg.
Citrate	$Na_3C_6H_5O_7$	1.0	−3	<1.0
CN^-	KCN	1.0	No color formed	<1.0
		0.2	−31	<0.2
ClO_4^-	$KClO_4$	25	−35	<25
		10	0	>10
IO_4^-	KIO_4	1.0	No color formed	0
MnO_4^-	$KMnO_4$	...	Reacts with reagent	0
$P_2O_7^{----}$	$Na_4P_2O_7$	5.0	−5	<6.0
		1.25	0	>1.25
S^{--}	Na_2S	0.1	−50	<0.1
Au^{+++}	$AuCl_3$	1.0	+3	<1.0
Pd^{++++}	$PdCl_4$	0.05	+4	<0.05
Pt^{++++}	H_2PtCl_6	1.0	+5	<1.0
Ag^+	$AgNO_3$	5.0	+3	<5.0

[a] Error in determination of 0.1 mg. of nickel.

Because tartrates are usually present in large concentrations for the purpose of complexation, extractions were carried out with large concentrations in the aqueous phase. Two grams of tartrate (47-ml. volume) caused no error. Benzoate (250 mg.) also showed no deleterious effect.

Copper present as a tartrate complex interferes by increasing the absorbancy of the system. The general shape of the absorption curve is not altered. Although copper may be separated from nickel dimethylglyoxime in chloroform with dilute ammonia solution (*1*, *9*), attempted removal of the copper α-furildioxime complex from the 1,2-dichlorobenzene solution by washing in this way was only partially successful. The ammonia concentration must be carefully regulated so that coextraction of the nickel into the ammonia solution does not occur. To test the effect of washing with ammonia, three 1,2-dichlorobenzene solutions containing the nickel complex of the same transmittancy were washed with 25 ml. (1 to 50), 10 ml. (1 to 10), and 25 ml. (1 to 10) of ammonia. The per cent transmittancy of the original solution was 29.0; after washing with 25 ml. of ammonia (1 to 50) the transmittancy was unchanged, but increased to 30.3 and 48.5% upon washing with 10 ml. (1 to 10) and 25 ml. (1 to 10) of ammonia solution, respectively. If the copper concentration is high relative to the nickel, the copper may not be completely removed from the nickel with the concentration of ammonia solution used. Therefore, results are best only when the amount of copper in the sample is known approximately. For highly accurate work it would be necessary to separate the copper in some more efficient manner.

Clean separations of some of the interfering ions which precipitate at a relatively low pH are not possible, owing to adsorption of the nickel on the precipitate.

DETERMINATION OF NICKEL IN ALLOYS

In order to test the reliability of the method, nickel was determined in a steel and a magnesium alloy.

Steel. N.B.S. steel sample No. 13c (containing an average of 0.196% nickel, 0.053% chromium, 0.165% copper, 0.700% manganese) was treated in the following manner:

A 1.000-gram sample was dissolved in 60 ml. of hydrochloric acid (1 to 1). After warming slightly to dissolve the metal, 10 ml. of nitric acid (1 to 1) were added, and the solution was boiled to expel the oxides of nitrogen, diluted to about 200 ml. with distilled water, cooled, transferred to a 500-ml. volumetric flask, and diluted to volume with distilled water. To a 25-ml. aliquot were added 5 ml. of 10% potassium sodium tartrate, 10 ml. of 3 *M* sodium acetate, 6 *M* sodium hydroxide to adjust the solution to pH 8.1, and 10 ml. of a 0.1% ethanolic solution of α-furildioxime. After extraction with 5-ml. portions of acid-free 1,2-dichlorobenzene until the organic layer was colorless, the organic phase was freed of water droplets by passing through a pledget of cotton in a funnel and the liquid was diluted to 50 ml. with fresh, acid-free 1,2-dichlorobenzene. The transmittancy of the solution was measured at 438 mμ, and the concentration of the nickel found from a calibration curve obtained by extraction of known amounts of nickel in a similar manner.

The percentage of nickel found in the alloy was 0.194 and 0.195, which is in close agreement with the average value of 0.196 as indicated by the National Bureau of Standards.

Magnesium Alloy. Nickel was determined in a magnesium alloy kindly furnished by the Dow Chemical Co., the percentage composition being aluminum, 6.35; copper, 0.058; iron, 0.033; nickel, 0.0024; manganese, 0.42; silicon, 0.177; and zinc, 2.38.

The sample (1.000 gram) was placed in a 250-ml. conical flask, 25 ml. of water were added, and then hydrochloric acid (1 to 1) in 5-ml. portions until complete dissolution of the metal. Iron was oxidized by boiling the solution a few minutes with 5 ml. of nitric acid (1 to 1). After cooling, 10 ml. of 10% potassium sodium tartrate, 5 ml. of 3 *M* sodium acetate, and sufficient 20% sodium hydroxide to adjust the solution to pH 8.0 were added. The solution was transferred to a separatory funnel, 15 ml. of a 0.1% ethanolic solution of α-furildioxime were added, and the nickel complex was extracted with 5-ml. portions of acid-free 1,2-dichlorobenzene. The layers of organic solvent were collected in a separatory funnel, and shaken with 5 to 10 ml. of dilute ammonia solution (1 to 50) to remove copper. The lower layer was then passed through a pledget of cotton in a funnel into a dry 50-ml. volumetric flask, and diluted to volume with the organic solvent. The transmittancy of the solution was measured as described previously.

The percentage of nickel found was 0.0024 and 0.0025, which is in good agreement with the value 0.0024, as given in the report accompanying the sample.

DISCUSSION

The main advantage of this method is that nickel is readily separated from colored solutions, such as iron or chromate, which would interfere in the usual method for determination of nickel with dimethylglyoxime. Because the complex in 1,2-dichlorobenzene or chloroform is sufficiently colored, it is not necessary to reextract the nickel into the aqueous phase, as must be done when either dimethylglyoxime or 1,2-cyclohexanedionedioxime is used as reagent in the extraction.

The narrow pH range necessitates careful pH regulation during extraction. The excellent stability of the complex is noteworthy in comparison with that of oxidized nickel dimethylglyoxime.

Salting-in effects of various electrolytes can be overcome by addition of sufficient sodium acetate.

If the amount of copper in a sample is unknown, it is advisable to separate the metal before determining the nickel. Small concentrations may be removed by washing the organic phase with a dilute solution of ammonia.

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Flow Colorimeter Adapted to Chlorine Analysis

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A flow-cell colorimeter finds manifold and increasing applications in industrial and laboratory chemical processes. The instrument described may be used with either gas or liquid samples, over a wide range of absorbancies. Optically it is a dual-beam type, using glass filters for spectral isolation. The simple line-powered electronic circuit gives meter indications which are approximately linear in concentration, and provides extra output for operating a recorder. The instrument combines accuracy with wide range of concentration measurement, simple and stable adjustments, and ruggedness and corrosion resistance to make it readily applicable to diverse problems. Use on plant streams containing chlorine gas is described.

FOR several years industry has needed a simple, versatile colorimeter capable of indicating, recording, or controlling chemical plant streams which are amenable to direct analysis by colorimetry. The Beckman Model DUR recording spectrophotometer has been used in applications where the wide wave-length range and other advantages resulting from employment of a high-resolution quartz monochromator and automatic standardization of either gas discharge or tungsten lamp source were required, but for many purposes a smaller and simpler instrument can meet all performance requirements.

The Beckman flow colorimeter is a reliable, compact, alternating current–operated instrument designed for industrial use. It employs a tungsten lamp source and glass filters for spectral isolation. A dual-beam arrangement with two phototubes minimizes the effect of lamp variations with line voltage. By employment of a novel multivibrator type of amplifier, the indication is made approximately linear with chemical concentration over a part of the meter scale.

Terminals provide an output suitable to operate a commercial potentiometer recorder or recorder-controller.

The instrument was designed to meet severe service requirements, such as are encountered in the analysis of chlorine gas streams. Provision is made for purging the case with clean air, and construction materials and finishes have been chosen with regard to their corrosion-resistant properties.

The instrument can be adapted for operation with any narrow-band filter combination commercially available, from 350 to 1000 mμ. For chlorine gas analysis it is fitted with Corning No. 5860 filters and 1P39 phototubes. A typical calibration curve of absorbancy (optical density) versus meter reading with this combination is shown in Figure 1. Concentration is nearly proportional to deflection over the first half of the scale, with compression at higher readings giving coverage of the entire concentration range on a single scale.

Because absorbancy is proportional to both concentration and path length, the indication may be varied widely by appropriate path-length selection up to the 10-cm. limit set for internal cells by over-all instrument size.

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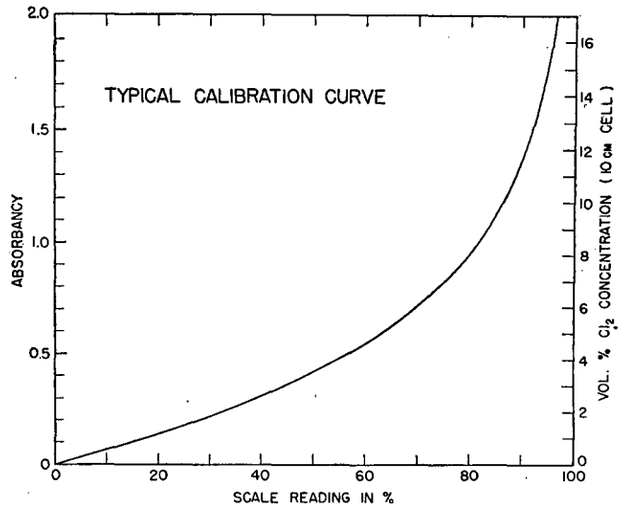

Figure 1. Typical Calibration Curve for Flow Colorimeter

The right-hand ordinate scale of Figure 1 shows the 10-cm. cell instrument calibration for chlorine in air at atmospheric pressure and room temperature. Under these conditions a partial pressure of 0.45 mm., or a volume concentration of 0.06% chlorine, will give 1% meter deflection.

From the slope of the curve of absorbancy versus scale reading it is easy to derive the variation of proportional analytical error (error in concentration divided by concentration) versus scale reading or absorbancy. This function is shown in Figure 2, for

an assumed constant reading error of 1% of full scale. Although this curve has a different shape from that for a conventional colorimeter or spectrophotometer similarly standardized (*2*), the minimum error factor and useful scale ranges are approximately the same for both instruments.

INSTRUMENT DESIGN

Optical System. This system is shown schematically in Figure 3.

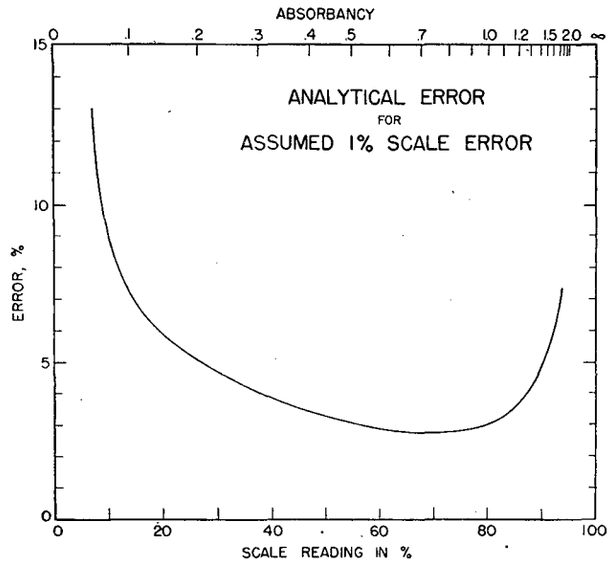

Figure 2. Proportional Analytical Error Factor for Colorimeter

It consists of a tungsten light source, which is an ordinary 32-cp. 6-volt automobile spotlight lamp, two photocells, and filters for the selection of the proper band of sample-illuminating radiation. In addition, a shutter and an adjustable diaphragm are provided for controlling the light to the photocells for purposes of preanalysis adjustments. The borosilicate glass condensing lens compensates for the difference in distances between the two photocells and the tungsten lamp, to improve optical efficiency and reduce the absorption cell volume.

The filter blocks are large enough to accommodate any standard Corning (*1*) narrow-band combination filter.

Amplifier. A circuit is used which, as shown in Figure 4, extends the symmetry of the optical system through the photometer, with the advantages of comparative simplicity of the power supply for alternating current operation, and freedom from critical components. In this circuit two amplifier tubes are used which conduct current in succession. The "on" time of one tube is limited by the current of the phototube in the reference beam, of the other by the phototube in the beam passing through the absorption cell. The phototubes serve to discharge a capacitor which determines the time interval each tube conducts. The circuit will thus be recognized as a variable-duty cycle multivibrator oscillator. The meter reading is unaltered by changes in

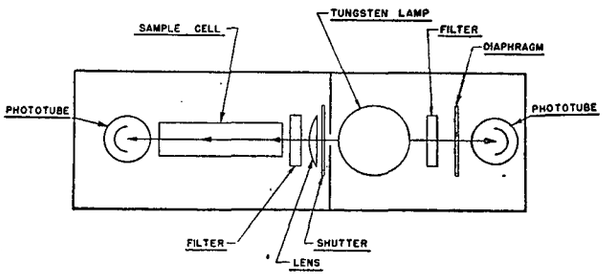

Figure 3. Schematic Optical Path of Colorimeter

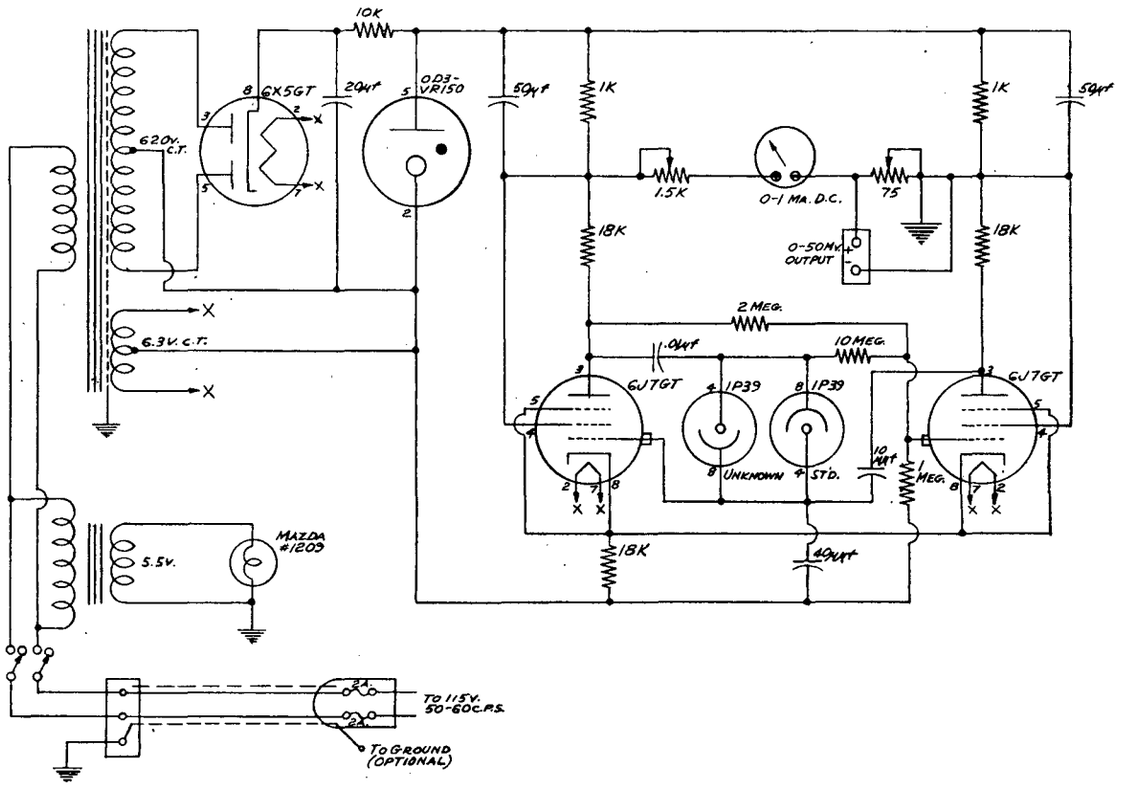

Figure 4. Wiring Diagram of Flow Colorimeter

lamp intensity, as each phototube responds in proportion, changing the frequency of oscillation but not the relative conduction time of each amplifier tube.

In the ultraviolet a change in line voltage alters not only the intensity, but also the spectral distribution of the radiation, causing a true change in the effective absorbancy of the sample, which results in a change of meter reading. This error is not serious, however, and it can be eliminated if desired by operating the colorimeter from a regulating transformer.

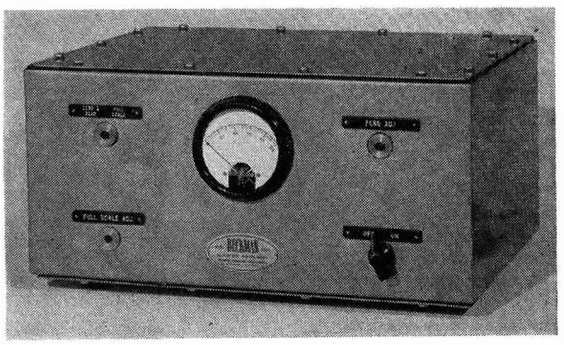

Figure 5. Panel View of Instrument

The operation principle described above explains the unusual form of the calibration curve of Figure 1. The conduction intervals of each tube are inversely proportional to beam intensities and approximately equal, so that no meter current flows when there is no absorption in the sample cell. The meter current will therefore be expected to be proportional to $\frac{1 - T}{1 + T}$, where T is sample transmittancy $\left(= \frac{1}{\text{antilog absorbancy}} \right)$.

The adjustable resistor located between the recorder terminals is for the purpose of setting the recorder potential to a value which brings the recorder and meter scales into coincidence.

Mechanical Design. The two principal objectives guiding the choice of construction materials and over-all mechanical design were resistance to corrosion and ruggedness. Gasket and connecting tubing are made of saran or neoprene, resistant finishes are used on the instrument case and parts, and moving parts are few and simple. Additional features which aid in preventing corrosion are the compartmentalized interior which serves to reduce the diffusion of any gases that might escape from the absorption cell connections, and the provision for purging the instrument case with an inert gas.

To minimize thermal drift within the cell compartment and to remove the heat of the lamp, a cooling coil has been placed around the lamp housing and on two walls of the cell compartment. Additional corrosion protection, and a material improvement in analytical accuracy result if the water for the coil is thermostated a few degrees above maximum room temperature with the aid of a constant temperature bath and circulating pump.

A panel view of the instrument is shown in Figure 5. The controls necessary for the operation of the flow colorimeter are few and require only occasional adjustment.

The slotted shaft in the upper right-hand corner of the instrument operates a metal diaphragm which is adjustable across the light path to the reference photocell. This control is set to zero the meter with a transparent material in the cell, after opening the shutter in the measurement beam controlled by the upper left-hand slotted shaft. The shutter may then be closed and the lower left-hand, electrical control adjusted to bring the output current to full scale, corresponding to complete absorption or infinite concentration. Alternatively, this control may be set with the shutter open to a chosen reading with a calibrating sample in the cell, to make the instrument approximately direct reading in concentration units at low absorbancy. The effect of such a calibration is to expand the curve of Figure 1 horizontally, up to 1.5 times.

Figure 6 shows the back of the instrument, where connections are made to the flowing sample, to the cooling water for maintaining thermal stability, to the source of 115-volt 50/60-cycle alternating current, and to a recorder or external meter where desired.

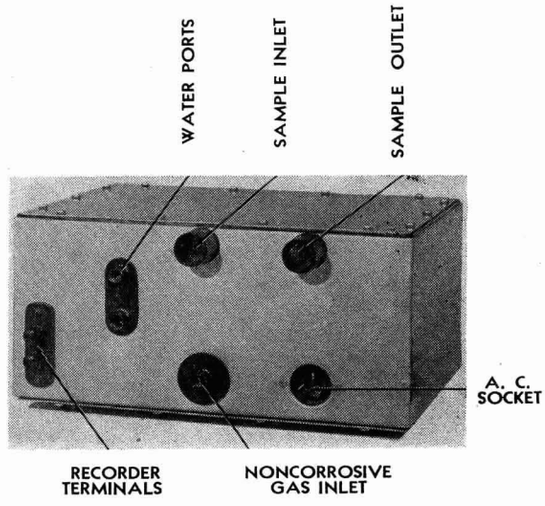

Figure 6. Rear View of Colorimeter

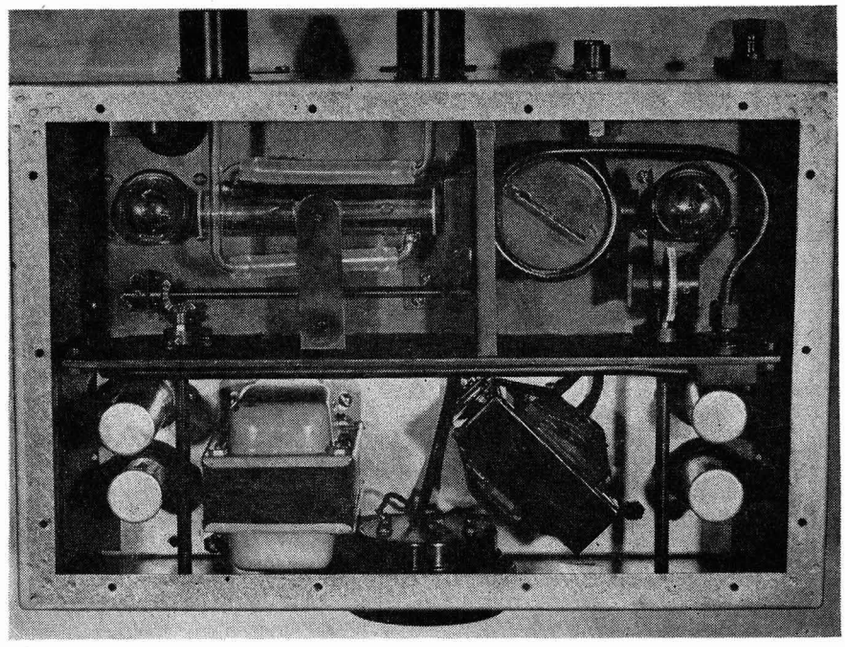

Figure 7. Top View of Instrument with Gasketed Cover Removed

Left. **Sample cell, phototube, filter block, shutter control**
Right. **Cooling coil, lamp housing, diaphragm, filter housing, diaphragm control**

The port marked "non-corrosive gas inlet" is for purging the interior of the instrument of corrosive gases from sample cell or environment. The outer case of the instrument is gasketed for further protection against dust and fumes.

Figure 7 is a photograph of the top of the instrument with cover removed, which shows more clearly the light traps on the glass tubing cell connections, the simple cell clamp, and the internal dividing walls, as well as the specially tubulated cylindrical Corex glass sample cell.

TEST RESULTS

Experimental tests of flow colorimeters set up for chlorine analysis showed the following results:

Response to line voltage change from 105 to 125 volts: zero shift, 0.2% of meter scale; full scale shift, 1.0%; shift with chlorine-air mixtures in cell approximately proportional to scale reading. These variations were effectively eliminated by supplying alternating current power from a regulating transformer.

Analytical accuracy. The first instrument was calibrated against known concentrations of chlorine in air, prepared by weighing liquid chlorine in glass ampoules, handling mixtures in glassware with phosphoric acid–lubricated stopcocks. After it had been in service for over 3 months on a chlorine-containing plant stream it was returned for a minor modification. A recheck of scale calibration showed no change outside the reading error of about 0.5% in concentration at mid-scale. This instrument has been in use for several years, with entirely satisfactory results.

Another instrument was extensively tested in the user's laboratory under carefully controlled conditions, preliminary to industrial application. Satisfactory performance was reported, and the instrument is being installed in the plant.

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PRECISION COLORIMETRY

Application to Determination of Manganese

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To provide a test of the transmittance-ratio approach to colorimetric measurements, the analytical determination of manganese was studied. The method developed utilizes periodate oxidation of manganese followed by a comparison of the unknown with a known solution of high absorbance. Data are presented which establish the limits of precision and accuracy of the method when applied to pyrolusite ores. These data show that a precision of a part in 1000 is easily obtainable. The principles of this approach are confirmed.

THE use of the permanganate ion in the colorimetric estimation of microquantities of manganese is well known and has been standard analytical practice for many years (*1*, *8*, *11–13*). Willard and Greathouse (*14*) found the necessary conditions for the quantitative oxidation to the heptavalent state. They gave complete directions for the colorimetric determination of manganese in steel and showed that permanganate was time stable in solutions of widely varying acid and periodate concentrations. A complete study of the absorption spectrum of this system was made by Mehlig (*10*) who used a recording spectrophotometer. He found that the system followed the absorption law, and that the permanganate ion was stable for at least 2 months in sulfuric, nitric, and phosphoric solutions. The effect of 56 common ions was investigated, and a few were found to interfere seriously although arrangements could be made to separate them during the course of the analysis.

The purpose of this study was to apply this reaction to the precise colorimetric determination of manganese. The method used in this investigation was one in which a high absorbance reference standard was compared with the unknown. A theoretical basis for this approach has been developed by Hiskey and colleagues (*6*, *7*) and applied in several instances by Bastian (*2*, *3*, *4*). The essential feature of this approach is that the relative precision of measurement increases linearly with the absorbance, provided the standard and unknown are nearly identical. There is a further requirement that the absorption law deviation be small in spite of the very high absorbance and the broad pass band which are required in this instance. A precision equivalent to that of volumetry was found for this system.

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APPARATUS AND REAGENTS

The instrument used was a Model DU Beckman spectrophotometer with Corex cells of 1-cm. thickness.

Reagents were concentrated C.P. acids and dry chemicals which were used without purification.

The sulfuric-phosphoric acid mixture was made by mixing equal volumes of concentrated sulfuric and 85% phosphoric.

EXPERIMENTAL

The first studies were devoted to determining the precision which might be achieved in a practical way with the apparatus at hand.

To make these measurements a stock solution of potassium permanganate, free of impurities, was prepared. A 1-liter volumetric flask was made up to mark with this solution, 10.00 ml. of water were added from a buret, and the solution was thoroughly mixed in the flask. The concentration ratio between the original solution and the diluted solution was thus 1.0100. Using the more dilute solution as a standard, the relative transmittance of the more concentrated solution was measured on the Beckman spectrophotometer. The instrument was set for maximum sensitivity. The transmittance of the more dilute solution was also measured against water. The two solutions were then diluted in the same way, and a series of pairs was obtained, the concentration ratios of which were known, but which were at different levels of concentration. The transmittance of the more concentrated was measured against the more dilute and the latter against water at each level.

Table I. Precision Obtained with Permanganate System

(α = 1.0100)

A_1	I_2/I_1	α	$\Delta\alpha/\alpha = \Delta c/c$
0.109	0.999	1.0047	53×10^{-4}
0.223	0.997	1.0047	53×10^{-4}
0.447	0.992	1.0080	20×10^{-4}
0.884	0.982	1.0089	11×10^{-4}
1.300	0.969	1.0106	6×10^{-4}
1.721	0.962	1.0098	2×10^{-4}

In this way a table of data was obtained of transmittance ratios as a function of the absorbance, A_1, of the standard (Table I).

The concentration ratio, α, was obtained using the relation $\log I_1/I_2 = A_1(\alpha - 1)$. From these data it is evident that the precision of the measurement increases with the absorbance of the standard, becoming, for the higher values used, better than 1 part per 1000.

The next step involved setting up a calibration curve for the concentration range from 50 to 60 mg. of manganese per liter. Bastian, Weberling, and Palilla (*4*) have already shown that the absorption law is followed in this range. The calibration in this instance was done as follows:

Stock 0.1 *N* potassium permanganate was standardized against National Bureau of Standards sodium oxalate according to the procedure of Fowler and Bright (*5*). Portions of this standardized permanganate solution were weighed into 250-ml. beakers. Each portion was diluted to 150 ml. and 15 ml. of the sulfuric-phosphoric acid mixture and 0.4 gram potassium periodate were added. The solutions were boiled, cooled, and diluted to 250 ml. in a volumetric flask. All dilutions were made in a single flask at the same temperature.

In this way five solutions were prepared ranging in concentration from 49.60 to 58.81 mg. of manganese per liter. The relative transmittance of four of these solutions was then determined against the one lowest in concentration. Corrections were applied for differences in cell path as indicated by Hiskey (*7*). Table II gives the results obtained on five separate occasions.

Table II. Data for Calibration Curve

Mn Soln., Mg./Liter	Δc, Mg.	Relative Transmittances 1	2	3	4	5	Av.	Av. Dev.	Relative Absorbance, A_1 ($\alpha - 1$)	Slope, $\Delta c/A_1$ ($\alpha - 1$)
49.60	...	1.000	1.000	1.000	1.000	1.000	1.000	...	0.0000	...
51.82	2.22	0.808	0.810	0.807	0.809	0.808	0.808	0.001	0.0924	24.03
54.28	4.68	0.634	0.647	0.651	0.643	0.640	0.644	0.005	0.1914	24.45
55.63	6.03	0.566	0.573	0.567	0.571	0.568	0.569	0.002	0.2449	24.62
58.81	9.21	0.419	0.417	0.427	0.422	0.422	0.421	0.003	0.3753	24.54
									Av.	24.41
									Av. dev.	0.19

The equation of the curve is as follows:

$$C = 24.41\ A_1(\alpha - 1) + 49.60$$

where

C = concentration of manganese in milligrams per liter

$A_1(\alpha - 1)$ = difference in absorbance between solution and reference standard—49.60 mg. per liter. The absorbance of the 49.60 standard is 2.21.

With the calibration completed and after some preliminary testing the following procedure was adopted:

Transfer an accurately weighed sample containing 250 to 300 mg. of manganese to a 250-ml. beaker and carefully add 10 ml. of concentrated hydrochloric acid. Heat for 5 minutes and then allow to cool. Carefully add 10 ml. of concentrated sulfuric acid, rinse down the watch glass and beaker, raise the watch glass and bring the solution to fumes of sulfur trioxide, and fume for 5 minutes. Allow to cool to room temperature, dilute to 100 ml. with water, and stir until all soluble salts are in solution. Transfer the contents of the beaker to a calibrated 1-liter volumetric flask. Dilute to the mark, shake thoroughly, and allow the insoluble material to settle.

Pipet 50 ml. of the clear supernatant liquid (calibrated pipet) to a clean 250-ml. beaker. Add 15 ml. of sulfuric-phosphoric acid mixture, dilute to 150 ml. with water, and add 0.4 gram potassium periodate. Bring to a boil and boil gently for 5 minutes. Keep near boiling temperature for 45 minutes and then allow to cool. Transfer to a 250-ml. volumetric flask, dilute to the mark, and mix thoroughly. The solution is now ready for colorimetric determination. It is recommended that all dilutions be made in the same flask. If this is not possible, the appropriate correction should be applied to the final concentration.

Measure the transmittance of the unknown solution against a standard solution of manganese or permanganate which has been treated as described in the preceding paragraph. The transmittance measurement is made at 526 mμ and at a sensitivity such that 0.1% transmittance is equivalent to 1 galvanometer division. Carefully decolorize the colored solution by dropwise addition of 0.01% hydrogen peroxide, being careful to add no more than 1 drop in excess. Measure the transmittance of this solution against the standard manganese solution which has also been decolorized.

The data are treated as follows:

I_2/I_1 = transmittance of unknown measured against standard
I_{20}/I_{10} = transmittance of unknown measured against standard, both of which have been decolorized
$A = \log I_1/I_2 - \log I_{10}/I_{20}$ = corrected absorbance of unknown

Read off the concentration of the unknown corresponding to A from the calibration curve, or calculate the concentration from the equation of the calibration curve.

Calculate the percentage of manganese as follows:

$$\%\text{Mn} = \frac{C}{W \times F \times 40}$$

C = concentration of unknown solution in milligrams per liter
W = weight of sample in grams
F = ratio of volume delivered by 50-ml. pipet to capacity of 1-liter volumetric flask

DETAILS OF EXPERIMENTAL TECHNIQUE

In order to obtain results of the order of precision of 1 part per 1000 a number of details had to be carefully noted. One of the most important was the condition of the Corex cells. These had to be free of scratches and perfectly clean. At the beginning of this work, the matter of cleanliness gave considerable difficulty until the following technique was worked out:

Remove organic materials with appropriate solvents. Rinse the solvent with acetone and remove the latter with water. Place the Corex cells in the bottom of a beaker and cover with acid permanganate–periodate solution. Heat over a water bath to 45° C. and allow to remain for 1 hour; then rinse thoroughly. If a brown film forms on the glass, remove with acidified sodium sulfite solution. This treatment will usually clean the cells so that no film of any kind is left inside or outside. Dry the outside walls with lintless paper or cloth. Do not attempt to dry the inside. Carefully inspect the outside walls each time a reading is made and wipe clean if necessary.

Special attention also had to be paid to all volumetric operations to ensure high precision. No great difficulty was experienced owing to temperature effects. As long as all the dilutions are made at the same temperature, high precision is obtainable. During the time of transmittance measurement the solutions should be kept within 2° C. of each other.

The above procedure was applied to the analysis of two standard samples of pyrolusite ores which were available. These included a National Bureau of Standards ore and a Gold Coast ore. They are ones that had been analyzed repeatedly by the authors according to the volumetric method of Lingane and Karplus (*9*)

Table III. Analysis of National Bureau of Standards Ore 25b and Gold Coast Ore

Ore	Manganese, % Volumetric	Colorimetric
N.B.S. 25b	58.35[a]	58.31
		58.41
		58.40
		58.36
		58.38
		Av. 58.37
Gold Coast	56.47 ±0.05[b]	56.53
		56.46
		56.51
		Av. 56.50

[a] Certificate value.
[b] Determined volumetrically in course of this research.

Table IV. Analysis of Manganese Ores
(Per cent manganese)

No.	Origin, Supplier	Volumetric	Colorimetric	Difference
64-44	English Hydrate	53.57	53.61	+0.04
156-47	Synthetic-E. J. Lavino	52.44	52.73	+0.29
157-47	Synthetic-E. J. Lavino	57.38	57.12	−0.26
162-47	Morocco-Bowring	59.89	59.74	−0.15
32-48	Activated-Asbury Graphite	54.79	54.62	−0.17
72-48	Synthetic-E. J. Lavino	45.91	46.04	+0.13
2-49	Caucasian-E. J. Lavino	56.19	56.22	+0.03
3-49	Montana-E. J. Lavino	45.19	45.33	+0.14

with a precision of 1 part per 1000. The results obtained on these samples are given in Table III.

The data in Table III indicate that a precision of about 1 part in 2000 is achieved.

The routine volumetric analyses of a group of commercial pyrolusites are compared with the colorimetric method in Table IV.

In this comparison portions of the same massive sample that had been previously ground and sieved were analyzed by the two methods. There is no systematic variation between the two methods and in all probability the differences are normal to the routine analytical operations involved. It is also likely that traces of colored impurities are not contributing to the absorbance of the sample since this would cause positive differences between the colorimetric and volumetric methods.

It seems reasonable to conclude, therefore, that manganese determinations may be effected by a procedure such as this with a precision equal to that of the volumetric methods. In addition, this method illustrates the high absorbance approach to colorimetry.

ACKNOWLEDGMENT

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Procedure for Collection of Isotopic Nitrogen

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AS A result of interest (*15*) in a reaction mechanism involving nitrogen in organic molecules it was found profitable to consider methods other than the one in common use (*11*, *12*) for the preparation and collection of N^{15}-enriched nitrogen.

The reaction studied involved a rearrangement (*13*) of a polynitrogen compound into an isomeric substance, by a mechanism involving group migration or by some other means. In order to establish the course of the reaction, the extent of N^{15} enrichment at a specific point in the rearrangement product had to be compared with the extent of N^{15} enrichment (2 to 4%) in potassium nitrite from which the original substance was prepared. The nitrogen at this point could be obtained as ammonium ion, and thus the reaction of nitrite ion with ammonium ion suggested itself.

The two equations

$$KN^*O_2 + NH_4Cl \longrightarrow KCl + 2H_2O + N^*_2 \quad (1)$$

$$KNO_2 + N^*H_4Cl \longrightarrow KCl + 2H_2O + N^*_2 \quad (2)$$

intimate that the enrichment in the nitrogen produced from each reaction should be the same, within limits which may be considered satisfactory for a mechanism study, where the enrichment in the nitrite of Reaction 1 and of the ammonium of Reaction 2 is the same. Data for reactions of this nature are given in Table I, where the nitrite and ammonium ions have comparable enrichments. The nitrite ion was obtained by lead reduction (*15*) of enriched potassium nitrate (available from the Eastman Kodak Co.), and the ammonium ion was obtained by quantitative reduction of the potassium nitrite (purified as silver nitrite and reconverted to potassium nitrite with potassium hydroxide) in potassium hydroxide solution by means of ferrous hydroxide (*1*). The ammonia liberated by this reduction was collected in dilute hydrochloric acid and recovered by evaporation as ammonium chloride.

APPARATUS

Nitrogen Generator. A borosilicate glass flask of convenient size is fitted with an adapter (see Figure 1) with an inlet tube

The work was undertaken to develop a convenient method for direct comparison of N^{15} enrichment in nitrite and ammonium ions. The technique resembles the Dumas procedure for ultimate nitrogen analysis, and the procedure has been worked out in detail for the reaction, $NH_4^+ + NO_2^- \xrightarrow{\Delta} N_2 + 2H_2O$. Obvious extensions to other nitrogen-producing reactions, such as selective deamination or the Dumas combustion, are discussed, and suggestions for suitable modifications are made. Such adaptations will make possible more extensive comparisons of N^{15}-enriched compounds, particularly of organic compounds with inorganic sources or *inter se*, than are now convenient, because of the requirement that nitrogen be obtained as ammonium chloride for analysis, and the procedure should broaden the field of study by means of tracer N^{15}. The apparatus is inexpensive and simple to operate, and the limiting factor of accessibility of a mass spectrometer should offer little difficulty.

entering its side wall and extending to the bottom of the reaction flask and an outlet tube attached to the apex of the adapter. (A 50-ml. flask is suitable, but smaller equipment is required throughout where samples of less than 0.001 mole are to be used. Corresponding adjustment in all values such as gas volumes and pressures must be made for operation on a semimicro scale.) This is the simplest form of equipment and is suitable for the reaction between nitrite ion and ammonium ion, as the latter may be mixed at room temperature without reaction. If this procedure be used for reactants where reaction occurs immediately upon admixture, a second type of adapter is suggested (Figure 2), which should carry in addition a 10/30 ⅋ joint to which may be fitted a dropping funnel carrying a capillary stopcock sealed to a capillary 10/30 ⅋ male joint. The delivery tube of the funnel should be so constricted that it will remain filled with liquid at all times and thus not constitute an air pocket.

Collection Apparatus. A standard nitrometer, preferably of alkali-resistant glass, of 50- to 100-ml. capacity is used. Both the inlet and outlet stopcocks are three-way, and the upper or outlet stopcock is surmounted by a short length of glass tubing. A 10/30 ⅋ male joint may be used in place of a rubber connection to the conditioning tube (Figure 1). The 50% potassium hydroxide in the leveling bulb need not be treated with barium oxide as in the Dumas procedure unless the work is to be done on a semimicro scale, in which case the solution is prepared as for Dumas ultimate nitrogen determination (*4, 10*).

Table I. Isotope Percentages

$NH_4^+ + NO_2^- \rightarrow N_2 + 2H_2O$

Enrichment Source	Obsd. Atom % N^{15} in N_2	Excess % N^{15} in N_2	% N^{15} in Ion	Excess % N^{15} in Ion	Av. % O_2[b]	Av. % CO_2[c]
Pure[d] N_2	0.362	0.00	0.362	...	0.15	0.00
Pure[e] N_2	0.362	0.00	0.362	...	0.01	0.01
KN^*O_2	1.32	0.96	2.28[f]	1.92[g]	0.01	0.01
N^*H_4Cl[h]	1.31	0.95	2.26[f]	1.90[g]	0.01	0.01
$N^*H_4N^*O_2$[i]	2.26	1.90	2.27[j]	1.91[g]	0.01	0.01

[a] Consolidated-Nier mass spectrometer, Model 21-201, used for isotope ratio measurements.
[b] Calculated from mass 32 peak.
[c] Calculated from mass 44 peak.
[d] Highly purified tank nitrogen, compares favorably with other natural N_2.
[e] Ordinary $KNO_2 + NH_4Cl$.
[f] Average value of $N^{15}\% \times 2 - 0.36$ (calculated).
[g] From preceding $N^{15}\% - 0.36$ (calculated).
[h] Prepared by quantitative reduction of preceding K^*NO_2 (*1*).
[i] Mixture of equivalent quantities of K^*NO_2 and N^*H_4Cl.
[j] Average value of $N^{15}\%$ in each of the two ions (calculated).

Carbon Dioxide Source. Most tank sources of carbon dioxide are as unsuitable for this procedure as for the Dumas procedure. A completely air-free source is essential. A modification of Hershberg's (*6*) apparatus is suitable for this procedure: powdered dry ice in a Dewar bottle closed with a single-holed rubber stopper through which passes a glass delivery tube in the form of a T, one arm of which is connected to a mercury-filled pressure regulator. The bottle should be filled 12 hours prior to expected use.

Apparatus for Filling Sample Tube (Cross Tube). A cross tube made either of ordinary borosilicate glass tubing or of capillary tubing (stronger, and smaller volume) carries a female 10/30 ⅋ joint on the upper and lower branches, and three vacuum-tight stopcocks, one just above the lower ⅋ joint, one on one horizontal branch, and a three-way stopcock on the other horizontal branch (Figure 1).

Conditioning Tube. Between the nitrometer and the cross tube is placed a tube (10 × 100 mm.) which serves to remove moisture and residual traces of carbon dioxide. The lower end contains a few millimeters of Dehydrite, which is separated from the bulk of the filling of Ascarite by a glass wool plug similar to those used to hold the entire filling in place (Figure 1).

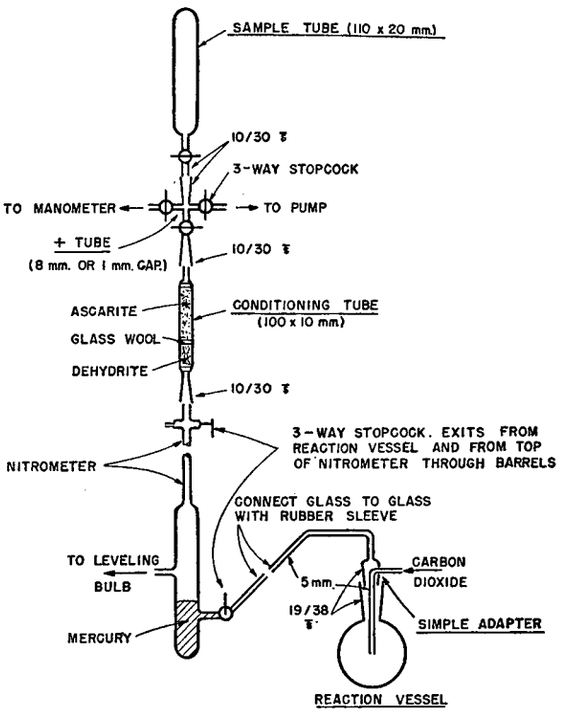

Figure 1. Diagram of Apparatus

Pump and Vacuum Gage. An efficient oil pump capable of rapidly evacuating the system to about 0.1 mm. is satisfactory for the scale of operation described. It is connected to the cross tube through the branch carrying the three-way stopcock. A vacuum gage capable of giving accurate readings in the region 0.01 to 1 mm. is connected to the cross tube through the other horizontal arm.

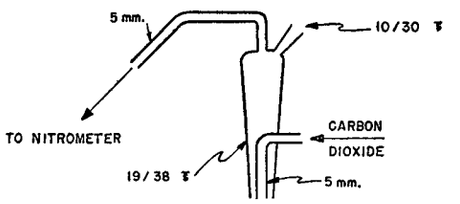

Figure 2. Special Adapter

Sample Tube. A convenient size of tube measures 20 × 110 mm. It is sealed at the top and is sealed to a 1- to 2-mm. bore capillary stopcock, which in turn carries a 10/30 male ⅋ joint, at the bottom.

The complete apparatus in position for assembly is shown in Figure 1. The nitrometer contains mercury to just above the level of the inlet tube to prevent a back-flow of the potassium hydroxide solution into the reaction vessel.

PROCEDURE

General. The literature abounds in directions for the Dumas type of ultimate nitrogen analysis (*2–5, 7, 9, 10*), and standard texts on organic micro- or semimicroanalysis include a good discussion (*4, 10*).

The nitrometer is filled with 50% potassium hydroxide solution prepared from water which has been recently boiled to render it reasonably free of nitrogen. Enough of the caustic solution is used to fill the tube completely and still be just visible in the leveling bulb. Fresh solution is advised after 10 to 15 determinations. Next the carbon dioxide source is tested for purity. The generator is connected directly to the nitrometer with the collection tube filled and the bulb at desk level. The gas is admitted to the tube at a rate not to exceed 2 bubbles in 3 seconds. When the bubbles shrink rapidly to micro size (about 0.2 mm. in diameter) and rise in the tube at a uniform rate without overtaking each other, the source is considered to be air-free. The entire apparatus is now assembled with the appropriate solution in the generating flask. Any solvents used in the reaction must have been rendered reasonably free of nitrogen by boiling and cooling in a nitrogen-free atmosphere, preferably carbon dioxide. All ⅌ joints should be sealed with Apiezon-W cement, all rubber connections wired and coated with glyptol resin, and all stopcocks lubricated with high-vacuum stopcock grease.

Procedure. The method was developed specifically for the reaction between nitrite ion and ammonium ion, but modifications suitable for other nitrogen-producing reactions are suggested.

Any conveniently small sample of ammonium chloride (0.05 to 0.25 gram) and an equivalent quantity of potassium nitrite are dissolved in about 25 ml. of water (previously boiled and cooled under carbon dioxide), and the solution is placed in the generating flask. Carbon dioxide from the previously tested source is now allowed to flow freely through the solution and flask through the adapter into the nitrometer tube (drained of solution) in order to sweep out all atmospheric gases. The stopcocks on the nitrometer are so adjusted that the gas passes out of the top without entering the conditioning tube. The sweeping operation requires about 15 minutes. The time will vary somewhat with the size of the apparatus and the rate of flow of carbon dioxide, and a few blank determinations should be carried out in order to determine the exact operating conditions.

When a suitable time has elapsed, the inlet stopcock of the nitrometer is closed, and the potassium hydroxide solution is drained into the measuring tube until it just reaches the base of the outlet stopcock barrel, which is then closed. At the same time the stopcocks on the cross tube are closed. The leveling bulb is lowered to desk level, and bubbles are admitted to the nitrometer at the rate of 2 bubbles in 3 seconds. If microbubbles are not observed, the sweeping process is continued until the train is free from atmospheric gases. When microbubbles are obtained, the source of carbon dioxide is shut off, and the inlet stopcock is gradually opened full, care being taken that the prescribed rate of inlet is not exceeded in the process.

The reaction flask is now heated by means of an oil bath or heating mantle at such a temperature that a steady stream of bubbles enters the nitrometer, 2 bubbles in 3 seconds. The volume of nitrogen which can be obtained in this way depends upon the quantities of nitrite and ammonium ion available, and formation of the gas falls off after about 60% (15 cc. at 25° from 0.05 gram of ammonium chloride) of the theoretical volume has been collected. The inlet stopcock is then closed. (A greater volume can be obtained by continued heating at a temperature below 90°, but it is generally convenient to discontinue the heating when the evolution of gas at this temperature slows down appreciably.)

An additional small volume may be obtained (before or after the extended heating) by opening the stopcock from the carbon dioxide generator full and then admitting gas from the reaction vessel to the nitrometer at the usual rate until microbubbles are obtained. The pressure of carbon dioxide must be sufficiently great to prevent a suck-back of the nitrometer solution into the reaction vessel, which might be caused by cooling and consequent condensation of water vapor with resultant drop in pressure. When the maximum volume of nitrogen has been obtained, the inlet stopcock of the nitrometer is closed to the tube, so that the generator is open to the air.

A modification of the foregoing procedure will be necessary if the reactants used to generate nitrogen react immediately upon mixing.

In place of the simple adapter, a modification (Figure 2) may be used. As before, all solutions or solvents should be freed of nitrogen before use. The appropriate solution is placed in the reaction vessel, which is then brought to the desired temperature—0° to 5° for selective deamination (*8*), for example. The other solution is placed in the dropping funnel, and the entire apparatus is swept out with carbon dioxide. After microbubbles have been obtained, the carbon dioxide is shut off, and the generator is ready for operation. The inlet stopcock to the nitrometer is opened full, and the solution in the dropping funnel is run in at an appropriate rate under a slight positive pressure of carbon dioxide. If gas is evolved at once, the addition is maintained so that the usual rate of gas flow is obtained. However, if the evolution of gas develops slowly on standing or upon heating, the solutions are mixed according to the conditions required by the reaction; if possible, the rate of gas flow is governed by the rate of heating.

Filling Sample Tubes. The sample tubes should be prepared in advance. They are first evacuated to as low a pressure as possible by the pump in use with the system. When the nitrometer has been filled and one is ready to collect the samples for mass-spectrometric analysis, a sample tube is fitted into the cross tube, a vacuum-tight connection being ensured by use of high-vacuum stopcock grease on the ⅌ joint. All stopcocks on the cross tube are then opened, and the system thus defined is evacuated to as low a pressure as possible with an oil pump (0.1 mm. is satisfactory). When the best vacuum possible has been attained, the sample tube stopcock is opened, and the stopcock leading to the pump is shut off. The system must be vacuum-tight at this point, as determined by previous tests.

Gas is now admitted to the evacuated system by very cautious adjustment of the outlet stopcock of the nitrometer until 5 cc. of gas have passed from the nitrometer tube. The nitrometer is then closed, and the entire system is re-evacuated. The flushing with 5 cc. of gas (approximately 100 mm. where the closed system is 35 cc.), followed by evacuation, is repeated, and in this manner any possible contamination by residual air is avoided. After the final flushing, nitrogen is admitted by similarly cautious adjustment of the outlet stopcock of the nitrometer until a sufficient sample for mass-spectrometric analysis is collected; a 5-cc. sample (atmospheric pressure) is adequate, and less may be used. If the sample tube is to be stored any length of time before analysis, less danger from atmospheric contamination will be encountered the nearer the pressure is to atmospheric. The nitrometer is then shut off, and the stopcock on the sample tube and all stopcocks on the cross tube are closed. The sample tube is freed by admitting air to that tube (not the pump!) through the three-way stopcock leading to the pump.

Additional samples may be collected by use of additional sample tubes. When a fresh tube has been seated, the three-way stopcock is closed to the atmosphere and opened to the cross tube system. The system is re-evacuated and the stopcocks leading to the manometer and pump are opened. The sample tube stopcock is now opened; and once the minimum pressure has been attained, the pump stopcock is shut off, the drying tube stopcock is opened, and gas is once more admitted from the nitrometer. In this manner several samples of the same isotope sample at the same or different pressures may be collected.

DISCUSSION

Table I summarizes data obtained for Reactions 1 and 2, where the enrichment of ammonium ion and of nitrite ion should be identical, because the former was prepared by quantitative reduction of the latter (*1*). In addition to the data recorded, there was also observed a significant peak at mass 30 which increased with time and temperature increase. That it was due to a side reaction producing nitric oxide was demonstrated by allowing air to enter a sample tube after analysis, whereupon a faint brownish tinge was observable. Other expected impurities, oxygen (which would imply spurious nitrogen, from the solvents or in leakage) and carbon dioxide (which would interfere by producing carbon monoxide, mass 28 and 29), are present in traces only, the recorded figures being the upper limits of contamination.

As an accuracy of better than 1% is possible in the range of enrichments recorded, it may be undesirable to use enrichments in which less than 1% excess N^{11} is present in the ions. For the range 1 to 4% enrichment the procedure has proved satisfactory; and modifications of the reaction producing the nitrogen are possible with little or no change in the method of generating and collecting the gas.

Undoubtedly, an equilibrium mixture, or nearly so, of N_2^{28}, N_2^{29}, and N_2^{30} is produced, as shown by the excellent agreement of the measured values of enrichment (2.26%) and excess N^{15} (1.90%) in $N^*H_4N^*O_2$ with the values calculated from the corresponding values for the individual enriched ions (2.27 and 1.91%). The calculation of the $\%N^{15}$ in the ions is based on the assumption that an equilibrium mixture is formed:

$$\%N^{15}\text{ (in ion)} = 2 \times \frac{100\,R}{2 + R} - 0.362$$

where R is the measured ratio for mass 29/mass 28. A slight variation noted in total N^{15} enrichment in individually analyzed fractions of nitrogen evolved (up to 75% of the theoretical) may be a real variation or an artifact, as may the difference recorded between the values for identically enriched ammonium and nitrite ions. These variations are at least in the right direction from a qualitative consideration of the relative zero-point energies of the isotopic ions.

Because the organic chemist interested in reaction mechanisms involving isotopic nitrogen is generally concerned with a difference in behavior between two very similarly situated nitrogen atoms, any process for preparing a sample of nitrogen gas from one without affecting the other should permit use of the general type of apparatus and procedure herein described. Where nitrogen gas may be evolved in a chemical reaction, as from two of three possible nitrogens in the same molecule, the nitrogen may be liberated and collected in the equipment described, or a suitable modification.

As a logical modification of the procedure, the use of the Dumas combustion for the preparation of the nitrogen sample is suggested. The danger of contamination with atmospheric gases in the potash solution (*11*) is no greater than in the present procedure. However, an improperly burned sample of organic compound may very well give rise to a fractional percentage of carbon monoxide. In order to guard against this possibility it is suggested that there be interposed between the combustion tube and the nitrometer a small tube filled with an iodine pentoxide—silica preparation (*14*) (preceded by a little Dehydrite) which will serve to oxidize any carbon monoxide to carbon dioxide. Nitrogen collected in this manner should be free from any significant quantity of carbon monoxide, and the method should be readily adaptable to micro work.

An attempt to produce nitrogen from α-amino acids by treatment with nitrous acid produced nitrogen contaminated with oxides of nitrogen and carbon monoxide (*11*). The use of an iodine pentoxide tube, between the reaction vessel and the nitrometer, is suggested for this reaction also.

Generally in isotope studies involving N^{15} the nitrogen has been prepared from Kjeldahl-produced ammonium ion by treatment with alkaline sodium hypobromite (*11*). This procedure is satisfactory for many purposes; however, if the source of nitrogen is other than ammonium ion, direct comparisons between source and product may not be feasible without conversion of the source to ammonium ion, which may not be desirable. However, the method has several advantages: The enrichment in the sample for analysis will be about twice that produced in the reactions (except the Dumas method) discussed above, the reaction producing nitrogen is quantitative, and the method is designed for micro scale operation.

The chief advantages of the present procedures are to be found in the much wider range of reaction type which may be used to obtain the nitrogen sample, including the possibility of direct examination of the enrichment in nitrite ion, and in the greater simplicity of equipment. All parts of the apparatus except the cross tube and sample tubes will be found in any organic laboratory in which analytical procedures are standard practice. The calculations of percentage enrichment are precisely the same as in the older method (*11*).

Although the experimental application of this procedure to other reactions producing nitrogen has not been explored, experience with the fundamental operations of generating, collecting, and preparing samples of nitrogen for mass-spectrometric analysis suggests these ideas and suggestions will foster further use of isotopic nitrogen in mechanism and metabolism studies not heretofore possible because of the previous requirement that the nitrogen be available as ammonium chloride.

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Potentiometric Method for Microdetermination of Iron

Extension of Use of Silver Reductor

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DURING investigation of another problem it was necessary to determine iron in amounts of 0.5 mg. or less with an accuracy of 0.3% or better. As very precise methods for the macrodetermination of iron are widely used, few difficulties were expected in finding a suitable and convenient micromethod, but a search of the literature revealed no such procedure.

Numerous colorimetric methods have been described—i.e., *o*-phenanthroline (*1, 7, 9, 14*), α,α'-bipyridine (*5, 11, 13, 20*), thiocyanate, ferriferrocyanide, and thioglycolate (*2, 3, 10, 15, 22*). These methods are applicable for the estimation of a few micrograms of iron, but the error is usually of the order of 1 to 3%, and they may be adversely affected by traces of other ions, such as phosphate.

Volumetric procedures have been reported which are much more accurate than colorimetric ones, though larger amounts of iron are required for each determination. The method of Mislowitzer and Schaefer (*12*), involving the reduction of ferric ion with an excess of titanous chloride and back-titration with standard potassium bromate, was found unsatisfactory because the end points were indefinite and the reaction with potassium bromate was too slow even at elevated temperatures. Other

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The need arose to determine iron in amounts of 0.5 mg. or less with an accuracy of 3 parts per thousand or better. A method was developed by which iron in amounts of from 0.27 to 0.5 mg. was determined with an accuracy of 2 parts per thousand. Ferric iron is reduced by means of a scaled-down Walden silver reductor, and the resulting ferrous iron is titrated with sulfatocerate to the potentiometric end point. The method allows the relatively easy determination of a few tenths of a milligram of iron with an accuracy not possible with other methods. The procedure should be useful for the accurate determination of iron in organic materials such as foodstuffs and proteins.

volumetric methods have been reported (*4*, *19*), but their average errors are greater than 0.3%. A most promising method was that reported by Edmonds and Birnbaum (*6*) employing the silver reductor previously described; it has been applied on a macro scale by Walden *et al.* (*21*). However, the smallest amount of iron determined by Edmonds and Birnbaum was 0.686 mg. and their average error was 0.5%. The present report concerns an improvement and extension of this method to 0.27 mg. of iron, with a study of the variables that become significant when it is applied to determinations on so small a scale. Subjective errors of the method have been reduced by the substitution of a potentiometer for an indicator in determination of the end point. This modification also eliminates the necessity of applying a blank correction.

EQUIPMENT

Silver Reductor. The reductor was substantially that described by Walden *et al.* (*21*) with appropriate changes in scale. Because Smith and Cagle's (*18*) method of preparing reduced silver was not practicable, the silver used in these experiments was prepared by reducing 3 grams of silver nitrate (Baker and Adamson, analytical reagent) as directed by Walden *et al.* (*21*), and was washed and transferred to the reductor tube; the resulting 1.9 grams of silver filled the reductor tube to a height of about 8 cm.

Beckman Model G pH Meter, together with a saturated calomel and a platinum extension electrode. The pH meter serves satisfactorily as a potentiometer.

Microburet. The one described by Shaffer *et al.* (*16*) was used. An acceptable Becton-Dickinson Yale syringe (2 cc.) was calibrated (*16*) by measuring the dimensions of the plunger; one unit of volume delivered from this syringe was 0.8092 ml.

Air-Driven Stirrer (Aero-Mix, No. 58970, Braun Corp., Los Angeles, Calif.). This stirrer was used to rotate the 50-ml. beaker in which the titrations were made. It was mounted in the inverted position with its shaft inserted into the bottom of a large rubber stopper, which in turn held the beaker snugly in a depression cut in its upper surface. Rotation of the beaker causes continuous and thorough mixing of the solution, the electrodes from the potentiometer acting as baffles.

REAGENTS

Sulfatocerate Solution. A stock solution of approximately 0.1 *N* sulfatoceric acid was prepared from ammonium hexanitratocerate (G. Frederick Smith, reagent grade) according to the directions given by Smith (*17*). The reagent used for titrations was prepared each day by diluting 5 ml. of the stock solution to 100 ml. with 0.5 *M* sulfuric acid. Both solutions were kept in a dark cupboard while not in use, as the diluted solution deteriorates in the light (*17*, page 19).

1 *N* Hydrochloric Acid. Baker's analyzed and Du Pont C.P. reagent concentrated hydrochloric acid (specific gravity 1.19, 0.00001 to 0.00002% iron) were used without distillation and were diluted to 1 *N* with freshly redistilled water. The specifications of the acid suggest that the 25 ml. of this reagent required in the reduction of an iron sample might introduce as much as 0.6 microgram of iron into the determination. However, blank determinations failed to detect significant amounts of iron from this source (see Table I).

0.5 *M* Sulfuric Acid was prepared by diluting Baker's analyzed concentrated sulfuric acid (specific gravity 1.84) with redistilled water.

Standard Iron Solutions. Three such solutions were prepared —Nos. 1 and 2 from Merck's reagent grade iron wire (minimum iron content 99.8%) and No. 3 from J. T. Baker's iron wire ("for standardizations," iron content 99.7%). After being thoroughly cleaned and dried, the samples were weighed and dissolved in a few milliliters of dilute sulfuric acid solution in 1-liter volumetric flasks, the calibration of which had been checked These solutions were diluted to the mark with distilled water and mixed; they contained 0.5116, 0.4805, and 0.4373 mg. of iron per ml. respectively.

OPERATION OF REDUCTOR

The procedure for using the reductor was essentially that described by Edmonds and Birnbaum (*6*). However, before making each analysis it was found desirable to wash the reductor with 100 ml. of 1 *N* hydrochloric acid solution in order to remove traces of hydrogen peroxide (*8*) which accumulate while the reductor is not in use. After the iron sample had been placed in the reductor, the reductor was rinsed with 25 ml. of 1 *N* hydrochloric acid solution in 2- to 3-ml. portions, and each portion was allowed to run into the solution to be titrated before the next portion was added.

Usually the rate of flow of solution through a freshly prepared reductor is satisfactorily rapid (10 to 20 ml. per minute) under gravitational force, but with continued use, the silver particles pack down and the flow is considerably retarded. A partial vacuum was employed to increase the rate of flow under these conditions, so that an unnecessarily long time was not required for the reduction. However, the titration value for a given iron sample was not affected by varying the flow rate of solutions through the reductor from 1 to 10 ml. per minute.

TITRATION OF SAMPLE

The beaker containing the solution of ferrous iron was placed in its holder, the electrodes were introduced, and the stirrer was started. When the voltage had become constant (ca. +400 mv.) addition of the sulfatocerate solution was started, and after each addition the syringe tip was rinsed with 4 or 5 drops of 1 *N* hydrochloric acid solution. The change in voltage, ΔE, per 0.100 unit of sulfatocerate solution was calculated and recorded after each addition and the voltage at which the maximum change occurred was taken as the end point. The end point was found to occur between +650 and +700 mv.; usually ΔE per 0.100 unit of sulfatocerate solution was 1000 mv. or greater and was produced by the addition of 0.002 unit of the titrating reagent.

REDUCTOR AND TITRATION BLANKS

A blank titration of 25 ml. of 1 *N* hydrochloric acid solution which had been passed through the reductor required about 0.020 to 0.030 unit of sulfatocerate solution. Previous investigators have reported that the silver reductor gives no blank (*21*) and that the blank found above disappears if ferric ions are present in the solution (*6*). Because this blank was variable and was presumably due to the occurrence of hydrogen

peroxide in the effluent solution (*8*), it was necessary to determine accurately the blank for the whole determination.

This was done by reducing samples of iron of various known amounts and then titrating them as above. The volume of sulfatocerate solution required for each sample was then plotted against the sample size and the linear curve thus obtained was extrapolated to zero iron concentration. The data obtained from these determinations are shown in Table I. From the straight line which best fits these data it is seen that the volume of sulfatocerate solution required at zero iron concentration is 0.0004 unit (0.0003 ml.). This is one fifth of the smallest division of the microburet, and it was therefore concluded that the over-all blank was effectively zero. The results obtained with the three standard iron solutions are in excellent agreement. From these data the average normality of the sulfatocerate solution was calculated; the average deviation from the mean was 0.2% and the spread of results was 0.8%.

DETERMINATION OF IRON IN BIOLOGICAL MATERIALS

As the main objective was the determination of iron in biological materials, it was necessary first to free the iron of the accompanying organic matter. The most rapid procedure was recognized to be a wet-ashing method involving digestion of the sample with concentrated sulfuric acid and decolorization by hydrogen peroxide, which was completely removed by fuming. This procedure was tested by oxidizing a sample of hemoglobin with 1 ml. of concentrated sulfuric acid and hydrogen peroxide. The clear, colorless residual solution obtained was diluted to 5 ml. with 1 *N* hydrochloric acid solution and passed through the silver reductor in the usual manner. The result of the titration of the reduced solution indicated that this highly acid solution containing considerable sulfuric acid had caused the reductor to cease functioning properly. As an alternative, the dry-ashing method of Hummel and Willard (*9*) was adopted and solutions of iron obtained in this manner from organic materials were found to be entirely suitable for analysis.

DISCUSSION

The procedure as outlined has been found to be satisfactory (average error 0.2%) for the determination of iron in the amounts indicated. However, attempts to determine 0.2 mg. or less of iron always resulted in low titration values. Possibly these results arise from the action of the small amount of hydrogen peroxide formed in the reductor (*8*) and the passage of the resulting ferric ions out of the reductor without again being reduced, as discussed by Edmonds and Birnbaum (*6*).

Throughout this work considerable care had to be used in order to obtain reductors that operated in a satisfactory manner. For example, a silver reductor prepared by following the directions of Walden *et al.* (*21*) does not necessarily function without producing an appreciable titration blank. During the work reported here and in subsequent analyses fifteen reductors were prepared, and eight of these performed satisfactorily. Not only have freshly prepared reductors sometimes failed to function properly, but those which have stood unused for a day or so may perform improperly. Gross failure of a reductor may be recognized by an initially low voltage (less than +360 mv.) of the reduced iron solution and by a change in voltage at the end point very much less than normal. A malfunctioning reductor gives a blank attributable to hydrogen peroxide, the formation of which is catalyzed by the presence of silver chloride (*8*). Treatments designed to remove silver chloride from unsatisfactory reductors by washing with dilute ammonium hydroxide (*8*) or by reducing with zinc in the presence of dilute sulfuric acid solution (*21*) have failed to improve their performance. Operations designed to remove occluded air have been more successful—for example, the reductor may be washed with large volumes of 1 *N* hydrochloric acid solution and the silver particles suspended in this solvent allowed to settle slowly. In view of these results, it is recommended that once the reductor is ready for use air should not be allowed to come in contact with the silver particles.

Table I. Determination of Total Blank of Procedure[a]

Mg. of Iron Used[b], *X*	Units[c] of Cerate Solution Required, *Y*	Units[c] of Cerate Solution Equal to 1 Mg. of Fe
0.5085 (Solution 1)	2.424	4.767
	2.412	4.743
	2.413	4.745
	2.408	4.736
	2.414	4.747
	2.412	4.743
	2.424	4.767
	2.412	4.743
	2.410	4.739
	2.420	4.759
	2.410	4.739
	2.410	4.739
	2.424	4.767
0.4776 (Solution 2)	2.272	4.757
	2.268	4.749
	2.272	4.757
	2.278	4.770
	2.280	4.774
	2.272	4.757
	2.266	4.745
0.4347 (Solution 3)	2.062	4.744
	2.068	4.757
	2.068	4.757
0.3051 (Solution 1, diluted 3 to 5)	1.446	4.739
	1.448	4.746
	1.446	4.739
	1.456	4.772

The equation of line of best fit calculated by method of least squares is $Y = 4.751\ X + 0.0004$.

[a] In other experiments 0.2707 mg. of iron required 1.282, 1.282, and 1.278 units of cerate solution (only three titrations made, precision 0.14%) and 0.2804 mg. of iron required 1.332, 1.328, 1.336, 1.332, 1.331, and 1.334 units (precision 0.15%).
[b] Volume of solution used in a determination was 0.994 ml.
[c] One unit of volume equals 0.8092 ml.

Experience obtained in preparing and testing reductors has led to the adoption of the following precautions for the preparation of a reductor:

Reagents used must be of analytical grade.

The surface of the copper strips used must be scrupulously clean.

During the reduction of the silver nitrate solution, stirring should not be so vigorous that large amounts of air are stirred into the solution; on the other hand, rapid stirring is essential because small silver particles are desired. These conditions are most easily achieved if the volume of the silver nitrate solution is relatively large and the copper strips are suspended well beneath the surface of the liquid.

Dilute (0.5 *M*) sulfuric acid should be used for washing the reduced silver free of copper ions, as sulfuric acid in high concentration has a detrimental effect.

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Separation of Iridium from Lead on a Micro Scale

Gravimetric Determination of Iridium

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The work was undertaken as a necessary preparation for a study of the distribution of iridium in the fire assay. Iridium and lead were separated by two methods: Lead may be removed as lead phosphate and iridium subsequently determined by hydrolytic precipitation, or iridium may be determined in the presence of lead directly by a new organic precipitant, 2-mercaptobenzothiazole. This paper adds to a general scheme of analysis of the six platinum metals obtained in fire assays of ores and minerals.

A STUDY of the efficiency of the fire assay of iridium required the accurate determination of a few milligrams of the noble metal in a lead button of approximately 30 grams. In the search for a suitable method to accomplish this, it was found necessary also to devise a micro scale separation of iridium and lead present together as their chlorides. The only suitable method in the literature which could be used for the latter was that outlined by Gilchrist (*3, 4*) and Holzer and Zaussinger (*6*) for the separation of certain noble metals from copper and other base metals. Shorter micro scale adaptations of their procedures which incorporated the gravimetric determination of iridium by 2-mercaptobenzothiazole were developed.

SEPARATION BY "COMPLEXING" IRIDIUM WITH NITRITE AND REMOVAL OF LEAD AS PHOSPHATE

The quantitative separation of 0.1 gram of lead taken as lead (II) chloride from 0.1 gram of each of palladium and platinum has been reported (*3*). The noble metal was held in solution as a complex nitrite while lead was removed by precipitation by sodium bicarbonate at pH 8. An analogous method for the separation of iridium and lead has also been described (*8*), although neither experimental data nor references to other publications were given. An attempt was made to separate small quantities of lead and iridium in this way, but without success. When a hot solution containing 10 mg. of iridium and 1 mg. of lead as chlorides and at pH 1 was treated with 0.5 to 1 gram of sodium nitrite, boiled for 1 minute, and then neutralized with sodium bicarbonate to pH 8, no lead was precipitated on boiling the sample or even after standing for 12 hours; under the same conditions, lead was easily precipitated from a solution containing lead chloride only. The pH of the lead-iridium solution was raised to 12 by the addition of dilute sodium hydroxide solution, but no lead could be precipitated. The fact that lead remained in solution in the presence of iridium has not yet been explained. Similar observations were made when oxalate was used instead of nitrite. It was found, however, that after iridium had been complexed by nitrite, lead could be precipitated as lead phosphate at pH 6.

Reagents. Solutions of sodium chloroiridate (Na_2IrCl_6) were prepared by a modification of the method described by Hill and Beamish (*5*). A weighed amount of spectrographically pure iridium sponge was placed in a porcelain boat together with ten times its weight of analytical grade sodium chloride; the boat and sample were placed in a silica tube through which a current of chlorine was passed at the rate of one bubble per second. The effluent chlorine was passed through traps containing 6 *N* hydrochloric acid. The apparatus was heated in a muffle at 700° C. for 18 to 24 hours, after which time the tube was removed from the furnace and the contents were allowed to cool in an atmosphere of chlorine. The boat was removed and the contents were dissolved in 0.1 *N* hydrochloric acid; dilute hydrochloric acid was also used to wash out the silica tube. The resulting solution was filtered through a Whatman No. 42 filter paper into a volumetric flask; the beaker and filter were washed well with the dilute acid and the solution was made up to volume. The pH of the stock solution was 1 or less.

The filter and residue were ignited at 650° C., reduced in hydrogen, and weighed. A blank was determined in a similar manner. The contents of the traps were evaporated to a small volume, transferred to a test tube or small Erlenmeyer flask, and tested for the presence of iridium by the method described by Pollard (*7*), which was sensitive to 0.005 mg. of iridium in 0.5 ml. of solution. In the preparation of a number of standard solutions, only once was iridium discovered in the traps; on this occasion the amount was found by comparison to be 0.01 mg. or less.

The standard solution was further checked by means of the procedure outlined by Gilchrist (*2*), in which the iridium present in a sample is determined gravimetrically by the precipitation of hydrated iridium dioxide at pH 6. The presence of iridium in the filtrate from the hydrated dioxide was ascertained by Pollard's method; 0.01 mg. or more of iridium in a filtrate could be detected.

Table I. Standardization of Solutions of Sodium Chloroiridate

Sample No.	Metal Taken	Metal Recovered	Test of Filtrates
	Mg.	*Mg.*	
A1	10.07	10.29	No color
2	10.07	10.28	No color
3	10.07	10.26	No color
B1	2.48	2.50	No color
2	2.48	2.51	Trace
3	4.96	5.00	No color
4	4.96	5.00	No color

The results of the standardization are shown in Table I. The fact that the weight of iridium recovered from a hydrolytic determination is slightly larger than the amount of metal taken (when microsamples are used) has been noted (*5*). The results of an investigation of this problem will be the subject of a forthcoming publication.

Aqueous solutions of lead chloride were prepared by dissolving weighed quantities of analytical grade lead chloride which had been dried at 110° C.

Solutions of sodium nitrite, disodium hydrogen phosphate, ammonium chloride, sodium bromate, and sodium hydrogen carbonate in water were filtered through a filter paper of close texture.

Procedure. Samples were prepared by adding 1 mg. of lead as lead chloride to a known volume of standard (sodium chloroiridate) solution. The volume of the sample was made up to 20 ml. with water; the pH of the resulting solution was 1 or less. The sample was heated to near boiling and 5 ml. of a 10% sodium nitrite solution were added for a sample weight of 10 mg. of iridium or less. The sample was boiled for 1 minute and then neutralized to pH 6 by the addition of 10% solution of sodium hydrogen carbonate; a 0.01% solution of bromocresol purple was used as an external indicator. One milliliter of a 0.1% solution of disodium hydrogen phosphate was added for each milligram of lead present and the sample was boiled for 5 minutes. In order to coagulate the finely divided lead phosphate precipitate, 10 ml. of 95% ethyl alcohol and 1 drop of a 0.5% calcium chloride solution were added to the cool mixture and this was then placed on a steam bath for 2 to 3 hours.

After the digestion period, the precipitate was filtered out on a Whatman No. 42 7-cm. filter paper and washed a few times with 1% sodium nitrite solution at pH 6. The filtrate and washings were set aside and the precipitate was redissolved by passing 2 ml. of 6 *N* hydrochloric acid through the filter and washing it a number of times with water. The solution was collected in the original beaker. The lead was then reprecipitated, using 5 ml. of sodium nitrite solution but only one half the volume of phosphate solution. The combined filtrates were acidified with 1 ml. of 12 *N* hydrochloric acid and evaporated to near dryness on a steam bath. Six milliliters of 6 *N* hydrochloric acid were added and the mixture was evaporated again to a moist residue. This procedure was repeated twice. Finally 2 grams of analytical grade sodium chloride were added to the sample and evaporation was continued to dryness.

It is important to avoid baking the residue during the foregoing operations. When baking occurs, the subsequent hydrolytic precipitation of iridium is not efficient and some iridium appears in the filtrate (Table II, set 1). This may be a colloidal phenomenon.

The residue from the evaporation was dissolved in water, 5 drops of 6 *N* hydrochloric acid were added, and the sample was digested on a steam bath to coagulate the silica. The mixture was filtered through a Whatman No. 42 filter paper; the beaker and filter were washed six times with 0.1 *N* hydrochloric acid. The iridium was precipitated as hydrated iridium dioxide and weighed as the metal.

Tests were made at various points in the procedure for possible losses of iridium. The final lead precipitate was redissolved in hydrochloric acid and tested by fuming with sulfuric and perchloric acids; the presence of lead did not decrease the sensitivity of the test, which was 0.005 mg. of iridium in a volume of 0.5 ml. Only occasionally could any iridium be detected here. The silica residue was also leached with hydrochloric acid and tested; in a few samples there was a small loss of less than 0.01 mg. The filtrate from the hydrolytic precipitation of iridium contained large quantities of sodium chloride and hence the detection of small quantities of iridium was difficult.

The filtrates together with the first portion of the washings were evaporated to dryness on a steam bath and the excess sodium bromate was destroyed by the addition of hydrochloric acid. The total residue was then transferred to a 50-ml. Erlenmeyer flask and evaporated to dryness. Eight to 10 drops of acid lithium sulfate solution and 2 or more drops of perchloric acid were added, the acids were fumed for a few seconds, and then the flask was cooled; iridium, if present, imparted a mauve coloration to the white salt. The smallest amount of metal that could be detected was 0.01 mg. The presence of iridium in this filtrate accounted for most of the small error in the procedure.

Spectrographic examination of some recovered iridium residues chosen at random showed the presence of only traces of lead when compared with samples of iridium metal taken through the same procedure, when the only lead added was that introduced through the reagents. The results are shown in Table II. The weight of metal taken is the value obtained on standardizing the solution by the hydrolytic method described earlier.

The method described above could be used to separate lead from a group of the platinum metals (including platinum, palladium, and rhodium) and not merely from iridium alone. However, it is somewhat laborious and the hydrolytic precipitation of iridium is subject to a small positive error. This is illustrated in Table I and is in general confirmed by numerous experiments in the authors' laboratory with other platinum metals. It was decided to investigate alternative methods for the separation and determination of iridium. It was found that 2-mercaptobenzothiazole did not precipitate small amounts of lead from an acetate solution, while iridium was completely precipitated.

GRAVIMETRIC DETERMINATION OF IRIDIUM WITH 2-MERCAPTOBENZOTHIAZOLE

No organic precipitant for the gravimetric determination of iridium has been recorded in the literature. Tests made in this laboratory some years ago on the precipitation of platinum metals with organic monosulfides (*1*) showed either partial or no precipitation of iridium. However, 2-mercaptobenzothiazole, tested at the same time, showed promise as a gravimetric reagent for iridium but because of the pressure of war work this research was not continued. Since that time this same reagent has been used by a number of workers for the determination of platinum metals other than iridium. Pollard used 2-mercaptobenzothiazole together with a reductant for the separation only of rhodium and platinum from iridium in a solution of the three metals (*7*).

Reagents. A standard solution of sodium chloroiridate was prepared and standardized, using the procedure described.

Technical grade 2-mercaptobenzothiazole was purified by recrystallization from ethyl alcohol. A dried sample of the purified product melted at 180.5–181.0° C.

Procedure. To a 5-ml. sample of sodium chloroiridate solution (which contained approximately 1 mg. of iridium per ml.) were added 10 ml. of glacial acetic acid, 1 ml. of a 20% ammonium acetate solution, and 25 ml. of water. The solution was heated almost to boiling and 10 ml. of a freshly prepared 1% solution of the organic reagent in 95% ethyl alcohol were added, together with a small piece of ashless filter tablet to minimize

Table II. Separation of Lead from Iridium by Phosphate

Set No.	Iridium Taken	Lead Taken	No. of Detns.	Av. Iridium Recovered	Av. Deviation	Error of Average	Test of Hydrolytic Filtrates
	Mg.	*Mg.*		*Mg.*	*Mg.*	%	
1	10.28	1	8	10.03	±0.11	−2	Strong color
2	2.50		2	2.50		0	No color
3	5.00	1	3	4.95	±0.02	−1	No color
4	5.00	2	1	5.04		+0.8	No color
5	5.00	3	1	4.68		−6	Strong color
6	4.99	1	2	4.96		−0.6	Trace of color
7	4.99	2	4	4.99	±0.02	0	Trace of color

Table III. Determination of Iridium in Presence of Lead with 2-Mercaptobenzothiazole

Set No.	Iridium Taken	Lead Taken	No. of Detns.	Av. Iridium Recovered	Av. Deviation	Error of Average	Test of Filtrates
	Mg.	*Mg.*		*Mg.*	*Mg.*	%	
1	5.02	..	4	5.02	±0.01	0	No color
2	10.03	..	3	10.03	±0.01	0	No color
3	15.05	..	4	15.03	±0.01	−0.1	No color
4	20.06	..	2	20.05	...	0	No color
5	25.08	..	1	25.04	...	−0.2	Faint color
6	...	1	...	−0.01	...	...	
7	4.95	..	2	4.95	...	0	No color
8	4.95	1	3	4.98	±0.02	+0.6	Faint color
9	4.95	2	2	4.96	...	+0.2	No color
10	4.95	3	4	4.93	±0.02	−0.4	
11	4.95	7	3	4.95	±0.04	0	Faint color

bumping. The sample was then boiled vigorously for 1 hour; during this time the iridium separated as a bulky orange precipitate. At the end of the hour (or before, if the volume of liquid in the sample became less than 20 ml.), the cover glass and the sides of the beaker were washed down with a hot solution 2% in ammonium acetate and 2% in acetic acid. The final volume of the liquid was 50 to 70 ml.

The sample was set aside overnight on a steam bath and then filtered through a Whatman No. 42, 7-cm. filter paper and washed with 100 ml. or more of hot solution 2% in ammonium acetate and 2% in acetic acid. After washing, the filter was dried under a heat lamp to remove the excess moisture and then transferred to a tared crucible. The filter was blackened in a muffle at 350° C., heated for 45 minutes at 650° to 700° C., cooled, reduced in hydrogen, cooled in hydrogen and nitrogen, and weighed. Saturated solutions of calcium nitrate were kept in the desiccator and in the balance case to maintain constant humidity. A blank, determined in a similar manner, was subtracted from the weight of the sample.

The filtrates and washings were checked for the presence of iridium by evaporation to dryness and digesting with concentrated sulfuric and nitric acids on a steam bath. The liquid was transferred to a 50-ml. borosilicate glass Erlenmeyer flask and fumed strongly over a burner until colorless. After cooling, 1 or 2 drops of 70% perchloric acid were added and the contents were reheated until fumes of perchloric acid appeared. On cooling again, a mauve color indicated the presence of iridium, 0.01 mg. of iridium in 0.5 ml. of the solution could be detected.

Qualitative observations showed that precipitation from an acetic acid solution is incomplete if much mineral acid is present. It was found that the optimum concentration of either hydrochloric or nitric acid for complete precipitation by the method outlined is 0.005 to 0.01 *N*. The length of time during which the sample is digested on the steam bath is also important. Low results were consistently obtained for a digestion time under 15 hours. A few tests were made in which the organic reagent was dissolved in glacial acetic acid; this solvent offered no advantage over 95% ethyl alcohol. In determinations made in the absence of ammonium acetate, the precipitate was more difficult to remove from the wall of the beaker.

If the concentration of the hydrochloric acid in the precipitating medium was greater than 0.01 *N*, a preliminary treatment was carried out in which some of the acid was neutralized by the careful addition of dilute sodium hydrogen carbonate solution. Alternatively, the sample was evaporated to dryness on a steam bath, the residue was dissolved in water, and the resulting solution was filtered. Blank determinations were made with each increase in the amount of reagent added. When the sample weight of iridium was increased, the weight of 2-mercaptobenzothiazole was correspondingly increased. If the sample weight of iridium was larger than 20 mg., there was increased difficulty in transferring and washing the bulky precipitate. In these cases the method offers no advantages over the hydrolytic precipitation of iridium. The results of the determination of iridium over a range of sample weights are shown in Table III.

DETERMINATION OF IRIDIUM IN PRESENCE OF LEAD

Samples containing known quantities of iridium and lead as their chlorides were treated as described above for the determination of iridium by 2-mercaptobenzothiazole. A number of the recovered iridium samples were examined spectrographically for the presence of lead. Only set 11 showed trace contamination with lead above that introduced through the reagents. The results from single precipitations are recorded in Table III.

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Cholesterol in Blood Serum

Studies of Microestimation as the Pyridinium Cholesteryl Sulfate

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THE most widely used method for the isolation of small amounts of cholesterol in naturally occurring substances prior to its estimation involves precipitation as the cholesterol digitonide (*3*). In 1936, Sobel, Drekter, and Natelson (*7*) proposed the isolation of such cholesterol as the pyridinium cholesteryl sulfate salt. The method they used, however, was relatively cumbersome and required exacting technique, involving the use of the hygroscopic solid pyridine sulfur trioxide as the precipitating agent.

The purpose of the present investigation was to develop a more practical procedure for the isolation of cholesterol as this sulfate salt. It was found, after considerable investigation, that when small amounts of cholesterol (about 0.1 mg.) are dissolved in 0.5 ml. of carbon tetrachloride, and 0.1 ml. of pyridine and then 0.25 ml. of a saturated solution of cholorosulfonic acid in carbon tetrachloride are added, at least 97% complete precipitation of the sulfate occurs in less than a half hour (see Table I), as determined by the colorimetric estimation of cholesterol remaining in solution. A longer time produced no increase in precipitation.

The reaction involved may be written as

$$ROH + ClSO_3H \xrightarrow{C_5H_5N} ROSO_3HC_5H_5N + C_5H_5NHCl$$

This investigation was undertaken to provide a derivative other than the digitonide for the isolation and determination of small amounts of cholesterol and possibly other sterols. Small amounts of cholesterol may be quantitatively isolated as the pyridinium cholesteryl sulfate. Cholesterol in carbon tetrachloride and pyridine (5 to 1) is treated with chlorosulfonic acid. The precipitate is washed with petroleum ether and evaluated colorimetrically by the Liebermann-Burchard reaction. Values of total cholesterol in blood serum are similar to those obtained by digitonin. Depending upon the method of extraction, free cholesterol values are the same as or less than with digitonin. The new method not only provides an alternative procedure for microestimation of cholesterol, but may serve to elucidate the various combinations in which cholesterol is present in the lipide extract of blood serum. The derivative may be useful in isolating α-sterols following removal of β-sterols with digitonin. In large scale sterol isolation problems, it may obviate need for the more expensive digitonin.

where R = steryl radical, and may be applicable to hydroxysterols other than cholesterol.

The results obtained in the determination of cholesterol in blood with this new method were compared with results obtained with Sobel and Mayer's (*8*) variation of the Schoenheimer-Sperry (*6*) technique, both of which involve precipitation of cholesterol as the digitonide. This reference method of Sobel and Mayer was modified so that, by reducing all volumes by half, smaller amounts of serum (0.1 ml.) could be used, and a spectrophotometric method was devised which could be applied without change to the colorimetric determination of cholesterol in the form of the sulfate, the digitonide, or uncombined cholesterol.

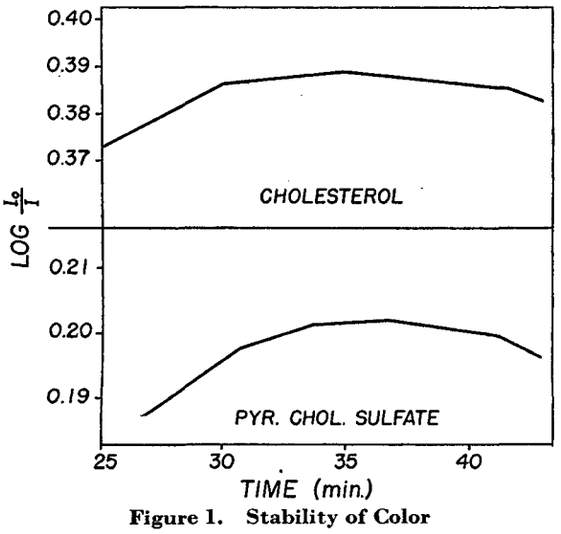

Figure 1. Stability of Color

Produced by action of 3 ml. of 25 to 1 acetic anhydride–sulfuric acid mixture on 1 ml. of acetic acid solution of cholesterol and pyridinium cholesteryl sulfate. Color developed in dark at 25° C., read at 625 mμ on Coleman spectrophotometer

The rate at which color developed after addition of color reagent to a solution of pyridinium cholesteryl sulfate in acetic acid was found to be about the same as that with cholesterol or cholesterol digitonide in that solvent (see Figure 1). Although it was verified that equivalent amounts of cholesterol and cholesterol digitonide give the same intensity of color (*6*), the color developed on the pyridinium cholesteryl sulfate was observed to be somewhat less than that from a corresponding amount of cholesterol or the digitonide. In all cases, maximum absorption was at 625 mμ.

All cholesterol values given in this paper were determined on the basis of cholesterol standards which were precipitated and treated in the same manner as the unknowns. However, a calibration curve for pure cholesterol (prepared by determining the color developed on standard solutions of cholesterol in acetic acid) gave essentially the same values for cholesterol which was precipitated as the digitonide as did those values based on the standards. This calibration curve, plotted in optical density against cholesterol in 1 ml. of acetic acid (up to 0.2 mg. of cholesterol), is a straight line in which 0.1 mg. of cholesterol gives an optical density reading of 0.50 when treated as described below. A sample of pyridinium cholesteryl sulfate of unknown purity was used to obtain a curve which similarly showed adherence to Beer's law.

In order to ascertain whether the digitonide and sulfate methods as used in this paper to precipitate cholesterol were suitable for the quantitative determination of precipitable cholesterol in blood serum, cholesterol in sera was determined both with and without the addition of known amounts of pure cholesterol. A difference in blood cholesterol values corresponding to the added cholesterol is evidence that precipitable cholesterol has been quantitatively determined. As seen in Table II, known amounts of cholesterol added to serum were quantitatively recovered as the pyridinium cholesteryl sulfate and cholesterol digitonide.

To test the sulfate method further, the cholesterol in extracts of saponified (*4*) sera was determined as the pyridinium cholesteryl sulfate, the cholesterol digitonide, and by direct colorimetric evaluation of the lipide extract (see Table III). The total cholesterol values obtained by the sulfate method were similar to the values obtained by the other two methods. Thus, the applicability of the sulfate method to total cholesterol estimation is indicated.

Table I. Precipitation of Cholesterol as Sulfate

(0.1 mg. used)

Reaction time, min.	5	10	15	20	30
Cholesterol precipitated, %	81.0	95.0	96.7	97.0	97.2

Table II. Typical Results in Recovery of Known Amounts of Cholesterol Added to Sera

(Determined as cholesterol digitonide or pyridinium cholesteryl sulfate)

Sample No.		Cholesterol Present	Cholesterol Added	Cholesterol Calculated	Cholesterol Found	Cholesterol Found/ Calcd
		γ/0.1 ml.	γ	γ	γ	%
A	1	40.0	40.0	80.0	77.6	97.0
	2	51.1	40.0	91.1	90.8	99.6
	3	47.0	50.0	97.0	99.0	102.0
	4	46.5	50.0	96.5	94.8	98.2
	5	37.6	40.0	77.6	77.1	99.4
B	6	39.3	50.0	89.0	88.7	99.7
	7	40.0	50.0	90.0	88.5	98.4
	8	32.3	50.0	82.3	84.0	102.0
C	9	191	200	391	391	100.0
	10	174	200	374	380	101.6

A. Cholesterol in serum as digitonide by method of Sobel and Mayer but reducing all volumes by half.
B. Cholesterol in serum precipitated as pyridinium cholesteryl sulfate. Results are typical for all methods studied for extracting cholesterol from serum. (Cholesterol was always added to serum before extraction in 0.1 ml. of solvent used for extraction. Volume of solvent subsequently added was correspondingly reduced.
C. Cholesterol in saponified serum precipitated as pyridinium cholesteryl sulfate after extraction with petroleum ether.

Table III. Cholesterol in Saponified Sera

Determined colorimetrically after precipitation as pyridinium cholesteryl sulfate and cholesterol digitonide, and directly on lipide extract. Values in mg./100 ml. serum)

Sample No.	Pyridinium Cholesteryl Sulfate	Cholesterol Digitonide	Direct Evaluation
1	164	164	158
2	185	185	192
3	188	192	192
4	266	265	268
5	270	277	278
6	271	278	283
7	284	287	281
8	377	384	380
9	843	835	833
Av.	316	319	318

The determination of "free" cholesterol as the sulfate is related to the basic problem of what free cholesterol is. In 1936, Drekter, Sobel, and Natelson (*2*) found that values for cholesterol in blood, determined as the pyridinium cholesteryl sulfate, were from 18 to 37% of the values obtained as the digitonide, although cholesterol added to sera was quantitatively recovered, within experimental error, as the sulfate. They postulated that free unesterified cholesterol, precipitated by digitonin, consists of "loosely bound" and "unbound" cholesterol, only the latter being precipitable as the sulfate salt.

Table IV. Ratio of Cholesterol Determined as Pyridinium Cholesteryl Sulfate to Cholesterol from Same Extract Determined as Digitonide

No.	Method of Extraction	Chol. as Sulfate/Chol. as Digitonide
1	Drekter *et al.*	0.18 to 0.37
2	Petroleum ether[a] / Alcohol-acetone	0.54 to 0.83
3	Alcohol-ether	0.59 to 0.71
4	10% H_2SO_4–CCl_4	0.52 to 0.84
5	50% H_2SO_4–CCl_4	0.38 to 0.90

Quantitative recovery of cholesterol added before extraction was obtained in all cases. All extractions were carried out at room temperature.

[a] Give identical results with individual bloods. Other methods of extraction not compared on same bloods.

Table V. Cholesterol Determined as Sulfate after Extraction in Alcohol-Acetone Mixture Evaporated at High Temperature, and as Digitonide

(Values given as γ/0.1 ml. of serum = mg. %)

	1	2	3	4
Pyridinium cholesteryl sulfate, heating alcohol-acetone extract	46.7	40.5	65.2	56.3
Cholesterol digitonide	46.2	43.7	61.8	54.5

Drekter, Sobel, and Natelson used three methods of extracting cholesterol from serum (two involving boiling alcohol-ether mixtures and the third involving extraction of serum dried on paper with a refluxing 3% solution of pyridine in alcohol), all of which gave essentially the same results. It appears, however, from the authors' data, that the ratio of unbound to free cholesterol depends on the method of extraction used (see Table IV). Although the results of these studies indicate a fairly wide range of unbound to free cholesterol ratios, they suggested that relatively drastic methods of extracting cholesterol from serum might break the loosely bound cholesterol combination which Drekter *et al.* postulated. Thus it might be possible to determine free cholesterol (digitonin-precipitable) by proper extraction followed by precipitation as the pyridinium cholesteryl sulfate. That such extraction methods are likely is indicated in Table V, which compares the cholesterol precipitated as the digitonide with that precipitated as the sulfate after extraction of the cholesterol by an alcohol-acetone mixture which is evaporated to dryness at high temperature.

The problem of the nature of loosely bound cholesterol will bear further investigation. In blood serum itself most of the cholesterol is in some combination. Only 3% of the cholesterol can be extracted with the same amount of petroleum ether that will completely extract cholesterol from the same serum previously treated with alcohol (unpublished experiments). Thus, free cholesterol is not really free in the blood serum, but is defined as free only by the fact that it is precipitated by digitonin in the lipide extract. In making this lipide extract, lipide-protein combinations are broken. It is possible that the combination of cholesterol with a compound (or compounds) that acts as a bridge to the protein still exists in the lipide extract. The possibility of such combination taking place during the process of lipide extraction is not excluded. Such combinations may be broken by digitonin as well as by evaporation of the acetone-alcohol extract at high temperatures (see Table V).

Bills (*1*) points out the many combinations which cholesterol is known to enter into in addition to fatty acids and esters. That loosely bound cholesterol is some such combination and is broken down by digitonin is suggested by the results of an experiment in which cholesterol digitonide precipitates were dissolved in pyridine (*5*), the digitonin was precipitated with ether, and the soluble cholesterol was determined as the sulfate (see Table VI).

The evidence that the sulfate method will quantitatively determine cholesterol as the free alcohol may be summarized as follows:

Results on saponified sera are similar to results by two other methods (see Table III).

Known amounts of cholesterol added to sera as the free alcohol are quantitatively recovered with all types of extraction employed (see Table II).

Following the precipitation of free cholesterol as the digitonide, the cholesterol in the precipitate can be quantitatively determined as the sulfate after splitting the cholesterol digitonide (see Table VI).

The estimation of serum cholesterol as the pyridinium cholesteryl sulfate should be of value:

To study the significance of free versus loosely bound cholesterol in health and disease.

To gain further insight into the cholesterol fractions of the organism.

As a procedure for estimation of total cholesterol in serum following saponification.

As a procedure for free cholesterol estimation, when time is an important factor. The time required for evaporation of the alcohol-acetone extract is 1 hour and for precipitation 0.5 hour. The total of 1.5 hours is distinctly less than the time required for the digitonin precipitation alone.

EXPERIMENTAL

Reagents (all analytical grade). Carbon tetrachloride. If on addition of redistilled chlorosulfonic acid the acid layer turns brown, the carbon tetrachloride should be washed with water, dried over anhydrous sodium sulfate, and redistilled.

Pyridine, redistilled.

Petroleum ether, 30° to 60°.

Standard solution of cholesterol in carbon tetrachloride, 20 mg. of cholesterol in 100 ml. (0.5 ml. of carbon tetrachloride contains 100 micrograms of cholesterol).

Alcoholic potassium hydroxide solution, 0.5 *N*. Dilute 3 ml. of stock solution (10 grams of potassium hydroxide in 20 ml. of water) to 50 ml. with aldehyde-free ethyl alcohol.

Alcohol-acetone mixture, 1 to 1 by volume of 95% ethyl alcohol and acetone.

Color-developing reagent (prepared freshly before use). Add concentrated sulfuric acid to ice-cold acetic anhydride in a 1 to 25 ratio and mix well.

Saturated solution of chlorosulfonic acid in carbon tetrachloride. Add sufficient chlorosulfonic acid (redistilled in all-glass apparatus) to carbon tetrachloride so that, after shaking, a layer of chlorosulfonic acid remains on the bottom of the solution. This reagent is stable but should be discarded when a brown color develops to any appreciable extent. Shake shortly before using to ensure a saturated solution.

Precipitation of Cholesterol as Pyridinium Cholesteryl Sulfate. To a solution of cholesterol in 0.5 ml. of carbon tetrachloride in a 15-ml. centrifuge tube 0.1 ml. of pyridine was added. The tube was shaken to mix the pyridine and carbon tetrachloride layers and to wash any pyridine from the tube walls. Then 0.25 ml. of a saturated solution of chlorosulfonic acid in carbon tetra

chloride was added and the tube was shaken immediately to prevent caking of the precipitate which forms at once. The tube was stoppered and left at room temperature for 20 minutes, during which time it was shaken once or twice. Then 5 ml. of petroleum ether were added and the mixture was shaken and then centrifuged for 10 minutes at 2000 r.p.m. The supernatant liquid was discarded, the precipitate was washed by shaking with 4 ml. of petroleum ether, and the centrifuging and washing were repeated. The precipitate was dried in a stream of nitrogen and dissolved at up to 60° C. in 1 ml. of acetic acid, which was added to wash the walls of the tube. If the acetic acid is not immediately added to the precipitate, the tube should be tightly stoppered to prevent absorption of moisture from the air. The precipitation mixture diluted to about 6 ml. contains 0.85 ml. of pyridine and carbon tetrachloride, in which pyridinium cholesteryl sulfate is soluble or slightly soluble. The use of more petroleum ether might therefore increase the 97% precipitation of cholesterol which is obtained.

Evaluation of Percentage of Cholesterol Precipitated as Sulfate. In a typical experiment 100 micrograms of cholesterol were precipitated in triplicate. The combined supernatant liquid and washings were evaporated to dryness at 40° in a stream of nitrogen. The cholesterol found in the residue (determined colorimetrically) was 8.6 micrograms or 2.87 micrograms lost in each tube. Thus the cholesterol precipitated was 97.13 micrograms per 100 micrograms of sample.

The validity of this method for evaluating losses was established by evaporating the supernatant fluid and washings of the reagents mixed in triplicate in the absence of cholesterol. Of 10.0 micrograms of cholesterol added to the resulting residue, 10.0 ± 0.18 micrograms were recovered in replicate estimations.

Cholesterol digitonide was precipitated by the method of Sobel and Mayer (*8*), using 0.1 ml. instead of 0.2 ml. of serum and reducing reagent volumes by one half.

COLORIMETRIC DETERMINATION.

Ten minutes after the color-developing reagent was mixed, 3 ml. of the reagent were added to 1 ml. of acetic acid solution of pyridinium cholesteryl sulfate, cholesterol digitonide, or pure cholesterol. The color was allowed to develop at 25° in the dark and after 35 ± 5 minutes was read in a Coleman Universal spectrophotometer set at 625 mμ with a Coleman PC.4 filter in place, using horizontal cuvettes of 5-cm. light path and 2.8-ml. capacity (*9*). Readings of unknowns were compared with those of a standard cholesterol sample containing 100 micrograms of cholesterol, which was precipitated and treated in the same manner as the cholesterol in the unknowns. Color-developing reagent only was used as a blank, as the addition of acetic acid did not alter results. With recalibration of the system, chloroform may be substituted for acetic acid.

Density readings made for this paper are the average of at least two determinations.

The precipitate of pyridinium cholesteryl sulfate fluoresces under ultraviolet light, so that a fluorometric method might replace the colorimetric method of determining cholesterol in this precipitate.

SAPONIFICATION OF SERUM

To 0.2 ml. of blood serum in a 6-inch round-bottomed test tube were added 5 ml. of 0.5 *N* alcholic potassium hydroxide solution. The tube was shaken and placed in a 45° oven for an hour. After saponification 5 ml. of petroleum ether and 3 ml. of water were added, and the mixture was shaken in the hand to mix the layers well. Then 0.5-ml. or 1.0-ml. aliquots of the petroleum ether were transferred into 15-ml. centrifuge tubes, evaporated to dryness, and taken up in 0.5 ml. of carbon tetrachloride, 2 ml. of 1 to 1 alcohol-acetone mixture, or 1 ml. of acetic acid, and the cholesterol present was determined colorimetrically as the sulfate, digitonide, or directly without separation from other lipides.

CHOLESTEROL EXTRACTION METHODS

Petroleum Ether Extraction (see Table IV). To 0.1 ml. of serum was added 0.1 ml. of alcohol and lipides were extracted with two 2-ml. portions of petroleum ether, which was then evaporated down. The extract was taken up in carbon tetrachloride and cholesterol was determined as the sulfate; and in alcohol-acetone cholesterol was determined as the digitonide.

Alcohol-Acetone and Alcohol-Ether Extractions (see Table IV). A 1 to 1 alcohol-acetone (*8*) or 3 to 1 alcohol (*8*) ether extract was evaporated down at room temperature and the extract was taken up in carbon tetrachloride for precipitation of the sulfate. Precipitation of the digitonide was carried out in alcohol-acetone.

Sulfuric Acid Extractions (10 and 50%, see Table IV). In a 3-inch test tube 1 ml. of 10% of 50% sulfuric acid and 1 ml. of carbon tetrachloride were added to 0.1 ml. of serum and the mixture was shaken for 20 minutes and centrifuged at 2500 r.p.m. for 20 minutes. The cholesterol in a 0.5-ml. aliquot of the carbon tetrachloride was determined as the sulfate and, after evaporation of the carbon tetrachloride, as the digitonide in alcohol-acetone.

Heating Alcohol-Acetone Extract (see Table V). To 0.1 ml. of serum in a 15-ml. centrifuge tube or 3-inch test tube were added 2 ml. of alcohol-acetone mixture to precipitate protein. The tube was shaken and centrifuged at 2000 r.p.m. for 10 minutes, after which the supernatant liquid was poured off into a 15-ml. centrifuge tube. The protein precipitate was washed with 1 ml. of alcohol-acetone mixture, and recentrifuged, and the washed liquid was added to the initial extract. The centrifuge tube containing the extract was placed in a hot water bath at 60° and the bath was heated slowly, so that 45 minutes later the temperature was 100° C. The tubes were allowed to remain in the 100° bath for 15 minutes, after which they were removed and allowed to cool, and the dried extract was taken up in 0.5 ml. of carbon tetrachloride. Pyridinium cholesteryl sulfate was precipitated as described above.

Table VI. Cholesterol in Sera

(Determined as sulfate from cholesterol recovered from digitonide precipitates, and directly as digitonide. Values given γ/0.1 ml. of serum = mg. %)

	1	2	3
Cholesterol digitonide	72.2	62.6	60.0
Cholesterol from digitonide determined as sulfate	75.2	59.4	58.6[a]

[a] Value from same serum after extraction in 50% H_2SO_4 and precipitation as sulfate was 31.6 with recovery of cholesterol added before extraction of 97.1%.

Determination of Cholesterol in Digitonide Precipitates as Sulfate. To the dry cholesterol digitonide precipitate from 0.1 ml. of serum 0.3 ml. of pyridine was added. After solution of the digitonide, 10 ml. of diethyl ether were added. The precipitated digitonin was redissolved in 0.2 ml. of pyridine after centrifugation and removal of the ether, and then reprecipitated with 5 ml. of ether. The tube was centrifuged and the ether extracts were combined, evaporated down, and taken up in 0.5 ml. of carbon tetrachloride, and the cholesterol present was determined as the pyridinium cholesteryl sulfate.

RECOMMENDED PROCEDURES FOR FREE AND TOTAL CHOLESTEROL

Total Cholesterol. To 0.05 to 0.1 ml. of blood serum are added 2.5 ml. of 0.5 *N* alcoholic potassium hydroxide solution. The contents of the tube are mixed and heated at about 45° for 1 hour. This is followed by addition of 1.5 ml. of water and 2 ml. of petroleum ether, after which the tube is shaken for half a minute by hand. One milliliter of the petroleum ether layer is removed and evaporated to dryness. The residue is dissolved in 0.5 ml. of carbon tetrachloride, cholesterol is precipitated as the sulfate, and color is developed and evaluated as described above.

Free Cholesterol. Free cholesterol is determined as described for cholesterol extraction methods, heating alcohol-acetone extract.

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Microdetermination of Chlorine, Bromine, and Iodine in Organic Compounds

Simplified Gravimetric Method

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A modified gravimetric method has been devised by which the chlorine, bromine, or iodine content of organic compounds may be determined quantitatively on a micro scale. Conventional microanalytical equipment is used. The presence of nitrogen, sulfur, and certain other elements causes no interference. The sample is burned in an atmosphere of oxygen using a platinum catalyst, and the halogen gases formed are absorbed by a silver gauze with the quantitative formation of the corresponding silver halides; the resulting gain in weight of the gauze represents directly the halogen content of the sample. In the presence of sulfur, the percentage of the halogens is calculated after silver sulfate has been removed from the absorbing silver gauze by water extraction. Samples with halogen contents ranging from approximately 10 to 97% have been analyzed successfully by this method.

THE quantitative microdetermination of chlorine, bromine, and iodine in organic compounds may be accomplished in a variety of ways. Reviews of the earlier literature and outlines of gravimetric and volumetric procedures for the estimation of these elements can be found in classical textbooks dealing with quantitative organic microanalysis (*14*, *15*). Excellent discussions of later advances in organic microchemistry were published not long ago (*2*, *6*, *19*, *20*). Because of certain attendant difficulties in many of the earlier analytical schemes, there has been a relatively recent increase in the number of publications devoted to new methods and modifications of existing procedures.

Metallic silver has been used for many years as an analytical reagent in quantitative organic microanalysis. Until recently, perhaps its major role has been that of a scavenger for removing interfering elements during the dry combustion of an organic compound. Lately, increased attention has been given to the use of silver as a primary reagent in the microdetermination of certain elements. As early as 1897, however, a procedure was developed by Dennstedt in which, during a carbon and hydrogen determination, tiny silver boats filled with molecular silver rested in the combustion tube to react with halogens and sulfur that might be in the sample (*5*). In the presence of oxygen gas, sulfur was presumably fixed by the silver as silver sulfate, while silver halides were formed directly. Subsequently, the silver boats were removed from the tube and weighed, and the silver compounds that had formed were dissolved in dilute potassium cyanide solution. Sulfate ions in this solution were determined gravimetrically with barium chloride. The difference between the weight of the silver boats following combustion and the weight of the sulfate gave the halogen content of the sample.

Following the original Dennstedt combustion procedure, Lacourt and co-workers (*11–13*) caught the single halogens (except fluorine) on silver gauze heated to 350° C. The resulting silver halides were put into solution, and precipitated as silver iodide with excess standard potassium iodide solution, and the potassium iodide not consumed was titrated iodometrically. These authors claimed that of the halogens, chlorine, bromine, and iodine, only chlorine could be determined directly from the gain in weight of the silver gauze through halide formation.

In an adaptation of the ordinary carbon and hydrogen microcombustion train, Balis *et al.* (*1*) carried out a simultaneous determination of carbon, hydrogen, and chlorine in gaseous organic compounds. An absorption tube which contained fine silver wire and electrolytic silver crystals at a temperature of 600° C. was attached to the exit end of the combustion tube. The chlorine content of a sample was estimated from the direct gain in weight of this absorption tube during combustion. The tube was said to require special handling in protecting the formed silver chloride from the light during cooling and weighing. In certain cases quantitative results were not obtained and the authors suggested that the fixing of chlorine by silver is not a simple combination reaction.

A somewhat similar procedure was used by Teston and McKenna in their simultaneous determination of carbon, fluorine, and chlorine in halocarbons (*18*). Chlorine (and bromine, if present) reacted with silver wire in an absorption tube maintained at 295° C. No interference from fluorine was reported and the chlorine and bromine analyses seemed accurate within ±0.9%. However, fluorine compounds containing hydrogen could not be analyzed since hydrogen fluoride apparently was formed; it etched the capillary tips of the tube containing the silver, thereby changing its weight. In addition, the relatively small surface area of the silver wire required that the absorption tube be repacked after every four determinations.

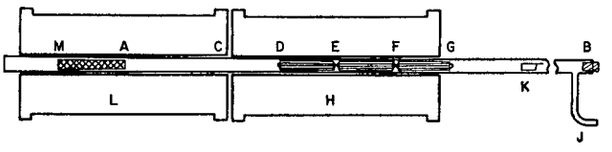

Figure 1. Combustion and Absorption Apparatus

For organic compounds containing iodine, Jurecek (*8*) decomposed the sample by catalytic oxidation and passed the combustion gases over a boat containing silver dispersed on magnesium oxide. The contents of the boat were then dissolved in dilute nitric acid and the silver iodide that had formed was filtered and weighed.

Following the Belcher and Spooner empty-tube combustion technique (*3*) almost exactly, Korshun and Sheveleva (*10*) have reported the analyses of samples containing carbon, hydrogen, sulfur, and the halogens (except fluorine). Silver ribbon placed in an absorption tube following the combustion tube, and heated to 450° C., retained the halogens. The limit of accuracy for the halogen analyses based on the weight gain of the silver was approximately 0.5%. Divided opinions have been expressed (*3*, *4*, *7*, *9*, *17*) concerning the general applicability of the unpacked- or empty-tube method of combustion. Undoubtedly further studies should be made to eliminate some of the difficulties that exist at present.

In view of the researches cited and results of earlier studies in this laboratory (*17*), it was believed that a simple, direct gravimetric method could be devised for the determination of chlorine,

bromine, or iodine through the reaction of these elements with silver to form weighable compounds. It seemed necessary to ascertain an appropriate temperature for the fixing of all three of these halogens, since for chlorine alone recommended temperatures ranging from 295° to 600° C. had been reported. Also, it was hoped that any procedure developed would not be too greatly restricted by the nature of the elements, other than the halogens, that might be present. A final desirable feature was to be the use of conventional microanalytical equipment. The method that evolved from these considerations is described herein.

APPARATUS

The essential features of the combustion and absorption apparatus are shown in Figure 1. Commercial tank oxygen is passed directly into a standard ACS pressure regulator (*16*) three fourths filled with saturated sodium carbonate solution, through a drying tube containing anhydrous magnesium perchlorate, and finally into a bubble counter containing 5% sodium hydroxide solution (these items of equipment are not shown) before entering the combustion tube. The latter is a 3-foot (90-cm.) length of standard wall Vycor tubing with an outside diameter of 13 mm. and a wall thickness of 1.2 mm. Side arm *J* is optional. The exit end of the combustion tube normally remains open to the atmosphere.

In this investigation, Fisher and Sargent microcombustion furnaces were used interchangeably for furnaces *L* and *H*. However, furnace *L* might well be of the standard ACS micropreheater type, in which case a standard Vycor microcombustion tube could be used after cutting off the conventional reduced end. The distance which the combustion tube protrudes from furnace *L* is not critical, except that its shortness will minimize possible loss from abrasion when the silver gauze roll at *MA* is introduced and removed. This metallic silver absorbent is a compact roll formed from an approximately 5-cm. square of 30-mesh wire gauze. In its preparation all protruding tips of silver wire are folded in, and the completed roll fits the combustion tube with moderate snugness but not so tightly that it cannot be removed easily with a platinum wire hook. A typical roll used in this work was 4.8 cm. long, had 3.25 turns, and weighed 4.7 grams. Before use, each gauze roll is cleaned and conditioned as described in an earlier publication (*17*).

The three platinum contacts at *DEFG* are the familiar ACS micro stars and are placed end to end so that contact *FG* extends about 1 cm. from the entrance end of furnace *H*. Normally a temperature of 650° to 700° C. is maintained at *E*, the hottest zone of combustion furnace *H*. During an analysis, the silver gauze roll is placed with one end at *A*, the center of absorption furnace *L*, while the maximum temperature of this furnace is held between 400° and 425° C. in order that the melting points of the formed silver halides will not be exceeded.

PROCEDURE

Furnaces *L* and *H* are brought to operating temperatures and a clean, dry, silver-gauze roll is weighed and positioned inside the combustion tube as indicated previously. A sample of approximately 3 mg. is introduced in platinum boat *K* and an oxygen flow rate of from 6 to 8 ml. per minute is established by attaching a Mariotte bottle at the exit end of the combustion tube and adjusting the pressure regulator as required. After the desired rate is attained, the Mariotte bottle is permanently disconnected and the rate of flow is checked periodically by observing the bubble counter.

Combustion is carried out with a movable burner, the rate of vaporization and burning of the sample depending upon the type of compound analyzed. The usual reburning operation is recommended, followed by a 10-minute flushing-out period to permit all products of pyrolysis to pass the silver gauze.

When combustion is complete, the gauze roll is withdrawn, transferred to a copper cooling block for 10 minutes, and then weighed. In the absence of sulfur, the gain in weight noted at this point (caused by halide formation) represents directly the chlorine, bromine, or iodine content of the sample. (Analyses based on subsequent extraction of the silver halides in various solvents have not proved satisfactory.)

When both sulfur and one of these halogens are present, the gain in weight of the silver gauze represents sulfate plus halide. For this case, following combustion and weighing, the gauze roll is placed in a beaker and covered with boiling distilled water for approximately 5 minutes to dissolve and remove the silver sulfate formed during combustion. The silver gauze is removed, washed thoroughly with distilled water, alcohol, and ether, dried directly in a standard microregenerating or drying block, and weighed after cooling. The loss in weight at this stage represents the weight of silver sulfate formed during the analysis; multiplication of this weight loss by the factor 0.3081 (SO_4/Ag_2SO_4) gives the weight of sulfate formed originally; this latter quantity subtracted from the total gain in weight of the gauze yields the halogen content of the substance analyzed.

After each analysis it is advisable to remove accumulated silver salts from the silver gauze. For silver chloride, immersion in 10% ammonia water for 5 minutes is effective, but for silver bromide

Table I. Analysis of Organic Halogen Compounds

Type Compound[a]	Elements Present	% Halogen, Theory	% Halogen, Found	Deviation of Average from Theory
Hydrocarbon	C, H, Cl	13.00	13.08, 12.85	0.12
Acid chloride	C, H, O, Cl	11.78	12.01	0.23
β-Chloroanthraquinone[b]	C, H, O, Cl	14.61	14.62	0.01
Ester	C, H, O, Cl	17.50	17.27, 17.29	0.22
o-Chlorobenzoic acid[b]	C, H, O, Cl	22.65	22.44	0.21
Ester	C, H, O, Cl	39.20	38.96	0.24
Chlorohydrin	C, H, O, Cl	55.03	55.09	0.06
Tar base	C, H, N, Cl	71.30	71.62	0.32
Organosilicon	C, H, Si, Cl	14.40	14.58	0.18
Amino acid deriv.	C, H, O, N, Cl	11.83	12.12	0.29
Peptide	C, H, O, N, Cl	13.65	13.73	0.08
Quinoline hydrochloride	C, H, O, N, Cl	16.13	15.91	0.22
Butyryl chloride	C, H, O, N, Cl	16.22	16.27	0.05
Peptide	C, H, O, N, Cl	21.16	21.26	0.10
Peptide	C, H, O, N, Cl	25.68	25.65	0.03
Aromatic sulfonyl chloride	C, H, O, S, Cl	16.21	16.23	0.02
Aromatic sulfonyl chloride	C, H, O, S, Cl	17.16	16.89, 16.89	0.27
Organosilicon	C, H, O, Si, Cl	20.00	20.20	0.20
Organosilicon	C, H, O, Si, Cl	30.10	29.84, 29.88	0.24
Acetylenic bromide	C, H, Br	42.30	42.18	0.12
Olefin	C, H, Br	43.19	43.42	0.23
Olefin	C, H, Br	74.71	74.84	0.13
Steroid ketone	C, H, O, Br	17.24	17.15	0.09
Ester	C, H, O, Br	21.64	21.48	0.16
Ester	C, H, O, Br	27.48	27.54	0.06
Ester	C, H, O, Br	28.13	28.14	0.01
Lactone deriv.	C, H, O, Br	28.13	27.91	0.22
Ester	C, H, O, Br	28.14	28.09	0.05
Ester	C, H, O, Br	29.60	29.47	0.13
Ester	C, H, O, Br	31.24	31.06	0.18
Steroid ketone	C, H, O, Br	32.60	32.61	0.01
o-Bromobenzoic acid[b]	C, H, O, Br	39.77	39.61	0.16
Bromohydrin	C, H, O, Br	73.33	73.49	0.16
2-Bromothiophene[b]	C, H, S, Br	49.02	48.89, 49.08	0.03
Porphyrin	C, H, O, N, Br	22.05	21.75	0.30
p-Iododiphenyl[b]	C, H, I	45.31	45.16, 45.00	0.23
p-Iodotoluene[b]	C, H, I	58.21	57.99, 58.20	0.11
Iodoform[b]	C, H, I	96.69	96.55, 96.63	0.10
o-Iodobenzoic acid[b]	C, H, O, I	51.17	51.16, 51.09	0.04
Steroid quaternary salt	C, H, O, N, I	21.00	20.92	0.08
Quaternary ammonium compound	C, H, O, N, I	32.40	32.11, 32.19	0.25
β-Diketone	C, H, O, S, F, I	36.46	36.62, 36.73	0.22
Hydrocarbon	C, H, Cl, I	53.70	53.41, 53.52	0.24
Hydrocarbon	C, H, Cl, I	58.10	58.42, 58.31	0.27
Not known	C, H, O, Cl, I	57.02	57.20, 57.01	0.09

[a] All the compounds are research samples except those marked with a superscript [b] which are standard compounds.
[b] Standard compounds.

and silver iodide the respective solvents are 10% sodium thiosulfate and 2% potassium cyanide solutions. Potassium cyanide is the best solvent for all the silver halides. Excess solvent and reaction products are removed by thorough washing with hot distilled water, alcohol, and ether. Before being used for another analysis, the gauze roll is placed in the combustion tube at operating temperature while oxygen is passed over it. After 10 minutes the roll is withdrawn, cooled, and weighed. This procedure of heating and cooling is continued until constant weight (±5 micrograms) of the roll is established.

DISCUSSION

Table I presents the results of the analyses of various known compounds for chlorine, bromine, and iodine, together with determinations on research materials. The values reported are based on the gain in weight of the silver gauze roll through silver halide formation. Exceptions are the three samples containing sulfur, for which the halogen contents were calculated by difference after silver sulfate had been removed by water extraction. Few duplicate values could be given for the chlorine and bromine analyses because, for the research compounds analyzed, only single determinations had been requested of the service laboratory. However, for the large number of compounds investigated the data indicate that the accuracy of the method is satisfactory for the determination of chlorine, bromine, and iodine in a variety of substances without interference from the other elements shown.

When a simultaneous determination of sulfur (*17*) and chlorine or bromine is attempted, one of these elements usually is found quantitatively at the expense of the other. The explanation lies in the fact that the lowest temperature, 450° C., recommended for the quantitative reaction of sulfur trioxide with the silver gauze to form silver sulfate is too high for complete retention of either silver chloride or silver bromide. At this temperature these compounds will volatilize in part from the roll, since silver chloride and silver bromide melt at 455° and 434° C., respectively. On the other hand, if one wishes to determine chlorine or bromine, the silver gauze must not be heated beyond, say, 425° C., and this temperature appears to be too low for quantitative absorption of sulfur trioxide. There should be no difficulty in determining sulfur and iodine simultaneously, because silver iodide decomposes at a temperature considerably in excess of 450° C.

Nevertheless, sulfur and these halogens can be estimated accurately in the presence of one another by carrying out two separate analyses. For the first determination, the silver roll is maintained at a temperature of 450° to 550° C. to favor quantitative formation of silver sulfate; this compound is then extracted in boiling water and the sulfur percentage is calculated. The second analysis is carried out with the silver gauze at 400° to 425° C. Any silver sulfate formed is extracted and the weight loss is converted to a weight of sulfate; the latter quantity when subtracted from the initial gain in weight of the gauze roll yields the halogen content quantitatively.

A further possibility for a simultaneous determination would involve the use of two silver rolls, the first being heated to 400° to 425° C. to react with all of the halogen and a portion of the sulfur (sulfur trioxide), and the second roll being maintained at approximately 500° C. to retain the rest of the sulfur. Thermostatic sleeves similar to those used to heat the lead dioxide reactant in a carbon and hydrogen determination would be suitable as furnaces to develop the various temperatures which are required.

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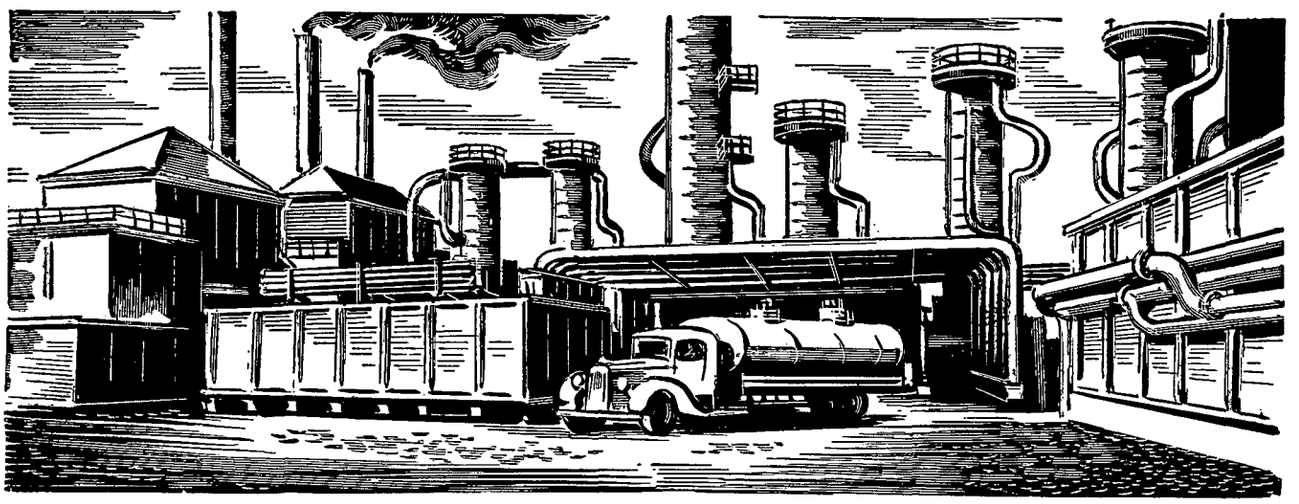


Recommended Specifications for Microchemical Apparatus

Micro-Kjeldahl Nitrogen

Committee for the Standardization of Microchemical Apparatus, Division of Analytical Chemistry, AMERICAN CHEMICAL SOCIETY

AL STEYERMARK, *Chairman, Hoffmann-LaRoche, Inc., Nutley, N. J.*
H. K. ALBER, *Arthur H. Thomas Co., Philadelphia, Pa.*
V. A. ALUISE, *Experiment Station, Hercules Powder Co., Wilmington, Del.*
E. W. D. HUFFMAN, *Huffman Microanalytical Laboratories, Denver, Colo.*
J. A. KUCK, *College of the City of New York, N. Y., and American Cyanamid Co., Stamford, Conn.*
J. J. MORAN, *Kimble Glass, Division of Owens-Illinois Glass Co., Vineland, N. J.*
C. O. WILLITS, *Eastern Regional Research Laboratory, Philadelphia, Pa.*

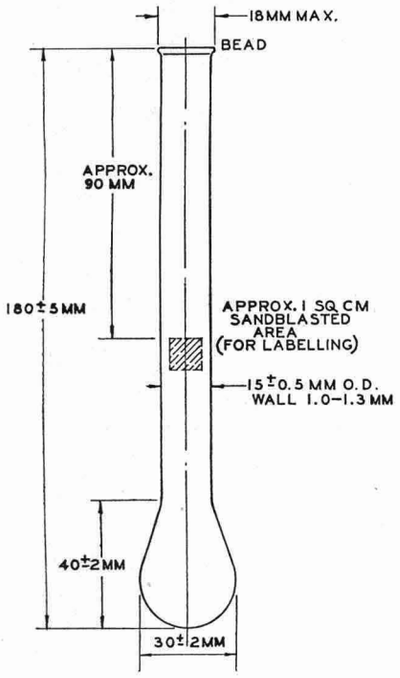


Figure 1. 10-Ml. Kjeldahl Flask

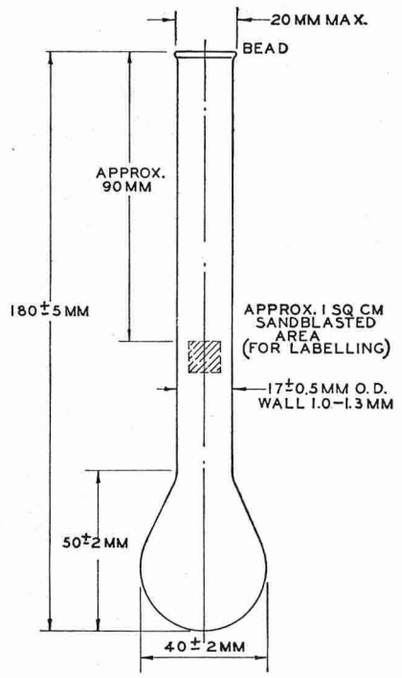


Figure 2. 30-Ml. Kjeldahl Flask

RECOMMENDED specifications for microchemical apparatus used in the carbon-hydrogen, Dumas nitrogen, halogen, and sulfur determinations (*2*) and the proposed program for the future (*1*) have been published. The present paper includes recommendations for the apparatus used in the Kjeldahl nitrogen determination and includes the following:

Kjeldahl digestion flasks, 10, 30, 30 (Soltys), and 100 ml.
Micro digestion rack
Manifold for micro digestion rack
Micro-Kjeldahl distillation apparatus, Pregl Type (Parnas-Wagner); electric steam generator
Micro-Kjeldahl distillation apparatus (one-piece model)

All recommendations have been made only after considerable experimental work by members of the committee.

Kjeldahl Digestion Flasks. Specifications for four types of flasks are recommended—10, 30, 30 (Soltys), and 100 ml. These are shown in Figures 1, 2, 3, and 4.

Micro Digestion Rack. The following specifications are recommended.

The micro-Kjeldahl digestion rack should be portable and consist of two parts, the flask heaters and the manifold support. The rack should provide for six Kjeldahl flasks, capacities 10 to 100 ml.

HEATERS. The source of heat may be either electricity or gas. The heaters must maintain a mixture of 2 ml. of sulfuric acid plus 1.30 grams of potassium sulfate at its boiling point (350° C.). The heaters must be sufficiently adjustable to provide for a mild digestion (low temperature) as well as distillation rates that will maintain refluxing of the acid into the neck of the flask. To avoid excessive heating of the flask necks, a shield is provided above the heaters. The shield should have circular openings not to exceed 26 mm. in diameter, directly above each heating element. The openings must be spaced 67 ± 7 mm. on centers. The rack must be provided with deflectors mounted under the burners to prevent overheating of the bench top.

Gas Heaters. The burners should be individually adjustable, with handles conveniently located at the front of the rack, and so constructed that they will be cool enough to manipulate even after long periods of operation. The burner must produce a nonluminous flame.

Electric Heaters. Electric heaters must perform satisfactorily at 100 volts. The controls must be conveniently located and should not become too hot to handle on continued op-

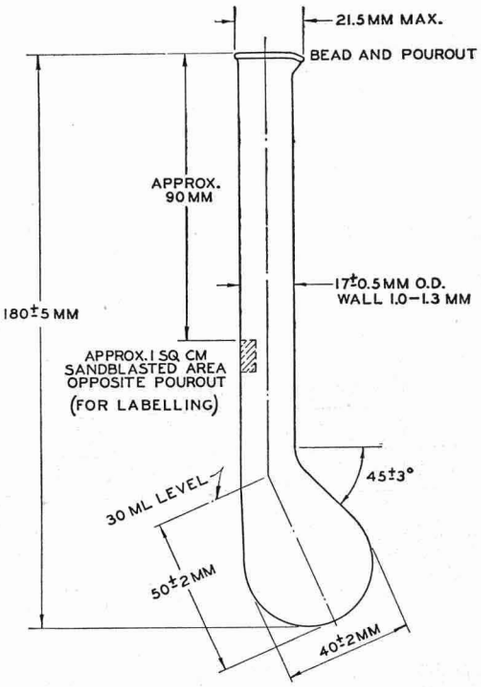


Figure 3. 30-Ml. (Soltys) Kjeldahl Flask

eration. Not more than two heaters should be operated from one set of controls.

MANIFOLD SUPPORT. This must hold the manifold securely, and also be adjustable, so that the manifold will support all the recommended sizes of flasks at the proper angle during digestion.

Manifold for Micro Digestion Rack. Figure 5 shows the recommended manifold. Any of the four types of Kjeldahl digestion flasks may be used with it.

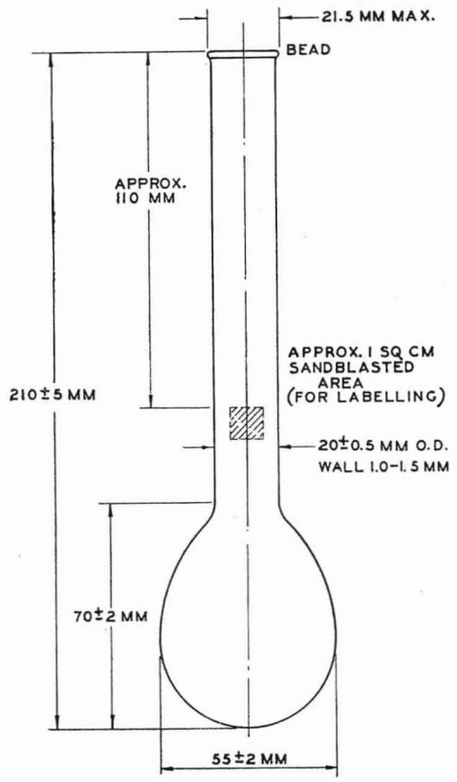


Figure 4. 100-Ml. Kjeldahl Flask

Micro-Kjeldahl Distillation Apparatus, Pregl Type (Parnas-Wagner). This apparatus, shown in Figures 6 and 7, is composed of the following parts:

Steam generator (2000-ml. capacity), *A*
Steam tube, *B*
Wiring assembly, *C*
Steam trap, *D*
Tube, *E*
Connecting tube, *F*
Distillation flask, *G*
Filling funnel, *H*
Condenser tube (glass or silver), *J*
Condenser jacket (West type), *K*
Drain, *L*

A new type of generator is recommended that provides closer control of steam generation than the usual gas-heated distillation flask. This all-glass steam generator, Figure 7*A*, is a wide-mouthed commercially available resin reaction kettle (Corning Glass Works, Corning, N. Y.). The interchangeable cover, with flat-ground rim and four ground-glass $\overline{\$}$ stopper tubulations,

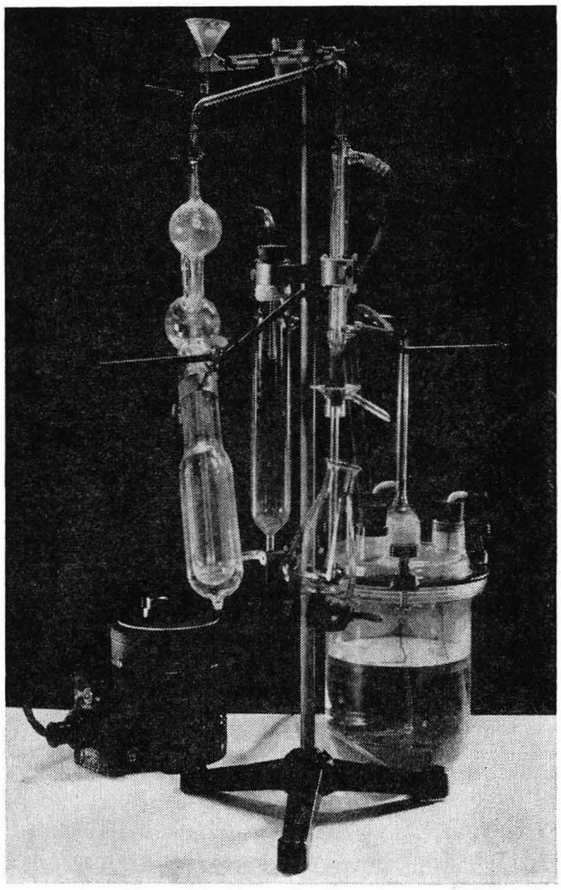


Figure 6. Micro-Kjeldahl Distillation Apparatus, Pregl Type (Parnas-Wagner)

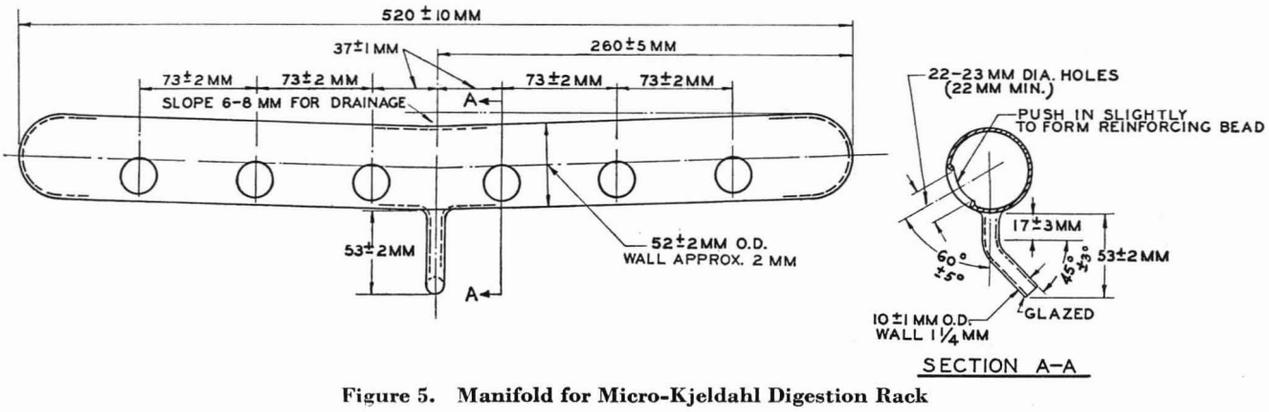
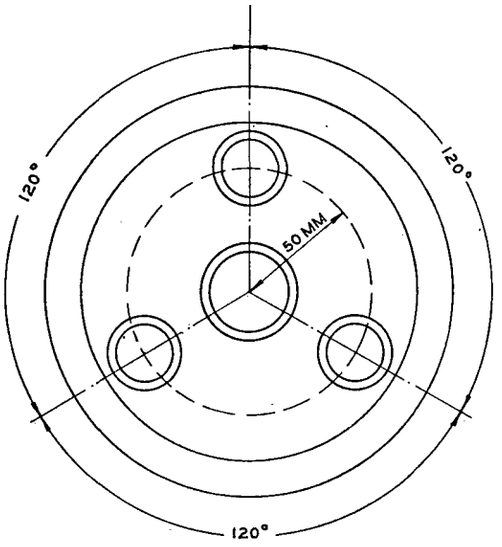


Figure 5. Manifold for Micro-Kjeldahl Digestion Rack



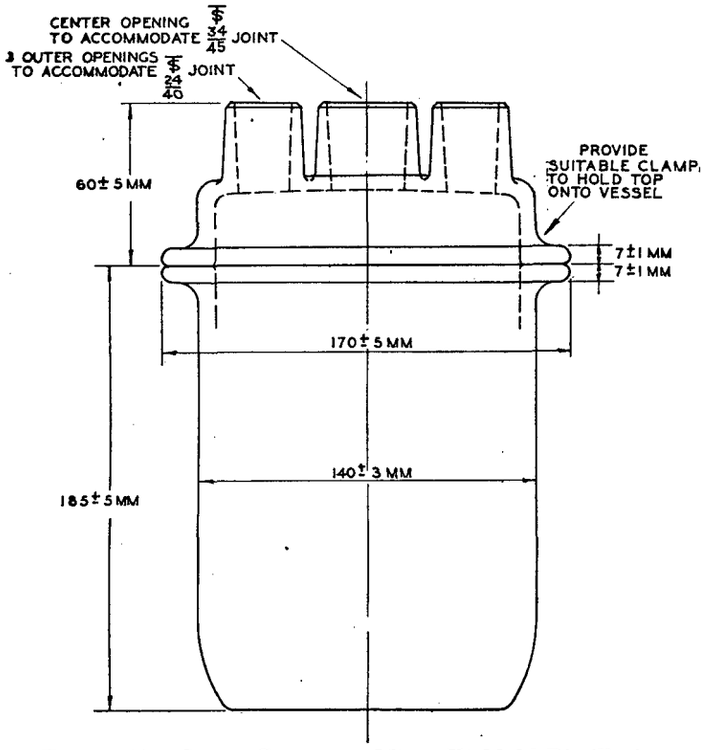


Figure 7A. Steam Generator, Micro-Kjeldahl Distillation Apparatus, Pregl Type (Parnas-Wagner)

is held in place with suitable clamps. To the center tubulation is attached the steam tube, Figure 7*B*. Two of the outer three tubulations are used for the leads of the electric heater assembly, Figure 7*C*. The remaining tubulation is used for a water inlet, or mounting a glass rod support for the resistance wire coil. The resistance of the heating spiral is about 16 ohms. The rate of steam generation is readily controlled by means of a 7.5-ampere variable autotransformer.

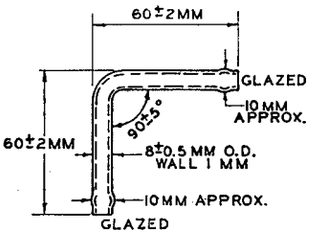


Figure 7F. Connecting Tube

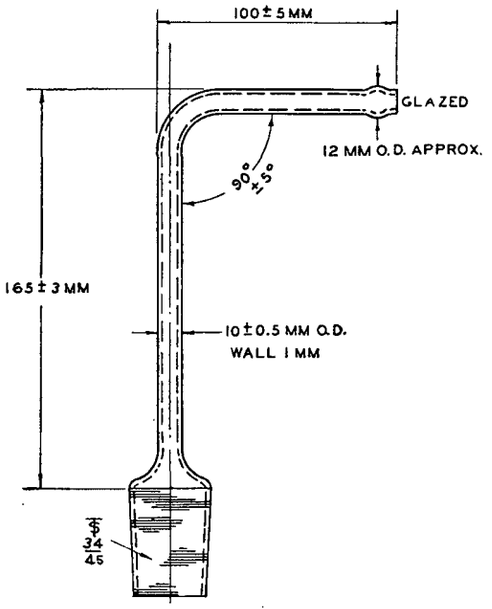


Figure 7B. Steam Tube

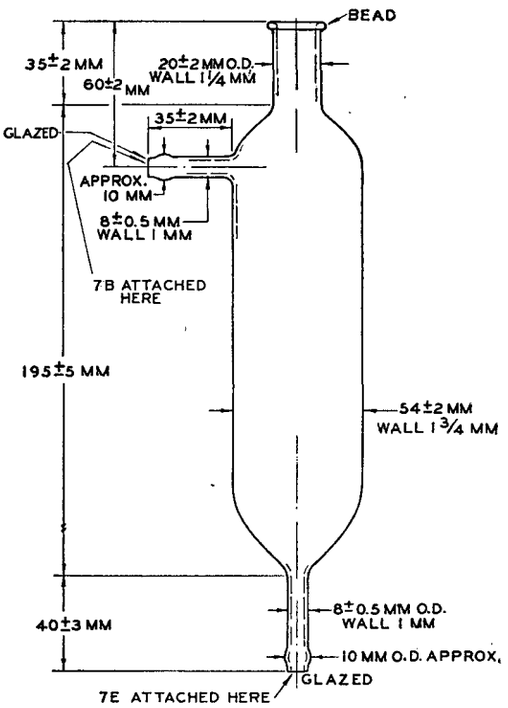


Figure 7D. Steam Trap

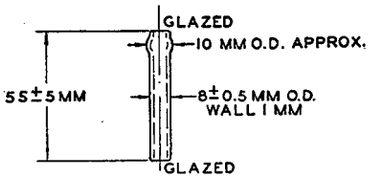


Figure 7E. Tube

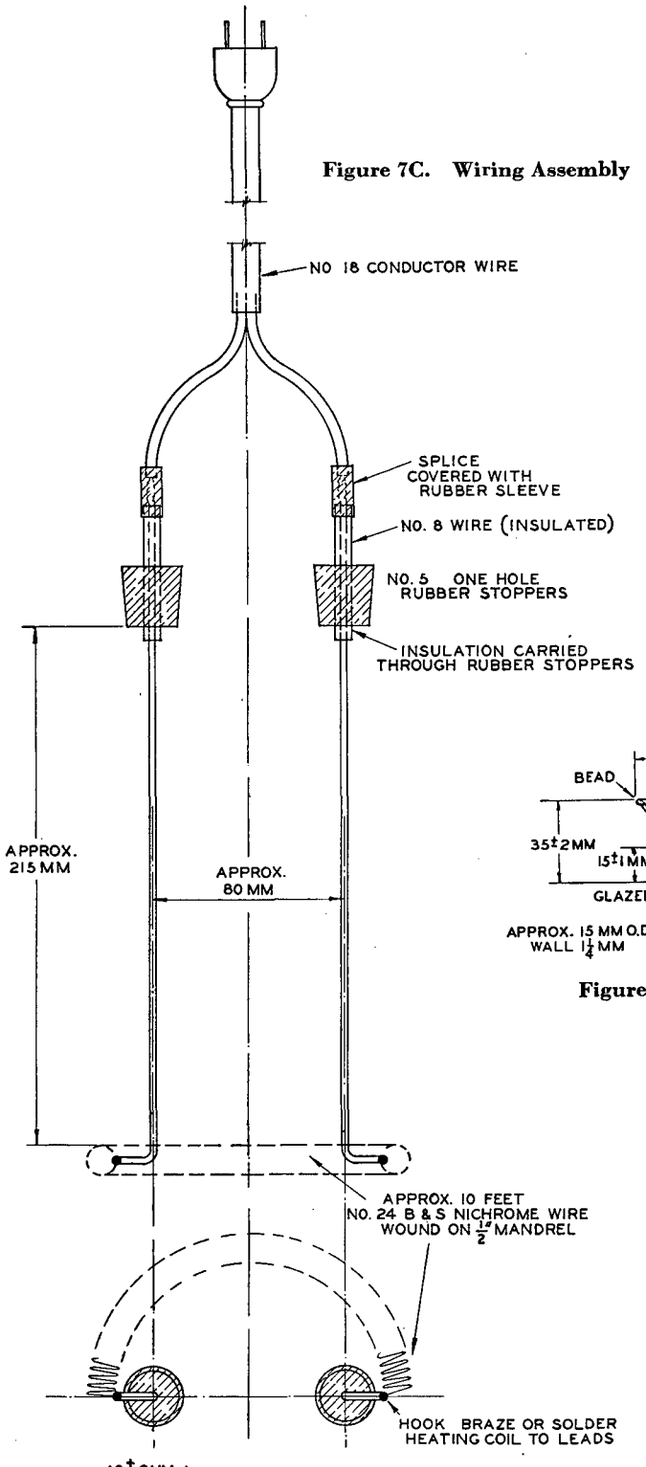


Figure 7C. Wiring Assembly

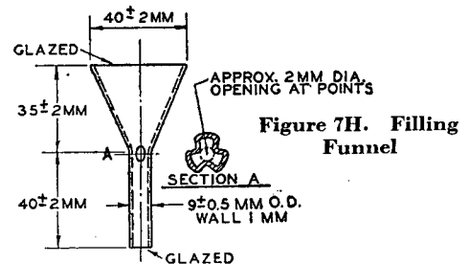


Figure 7H. Filling Funnel

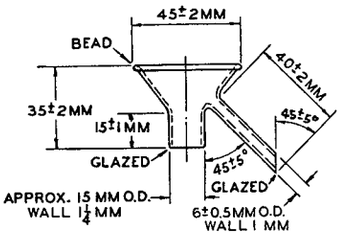


Figure 7L. Drain

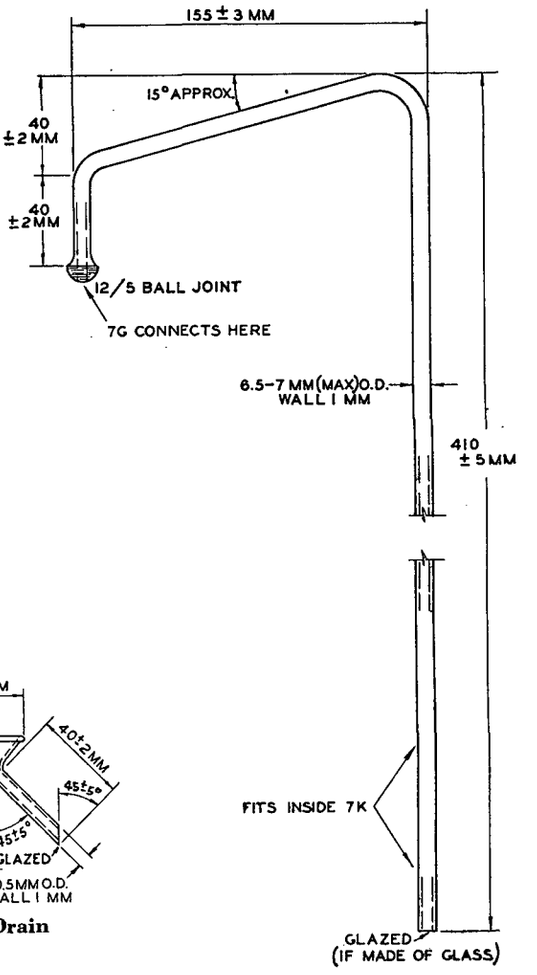


Figure 7J. Condenser Tube

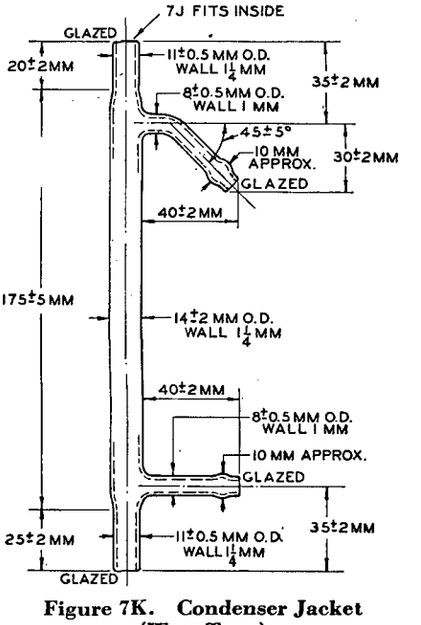


Figure 7K. Condenser Jacket (West Type)

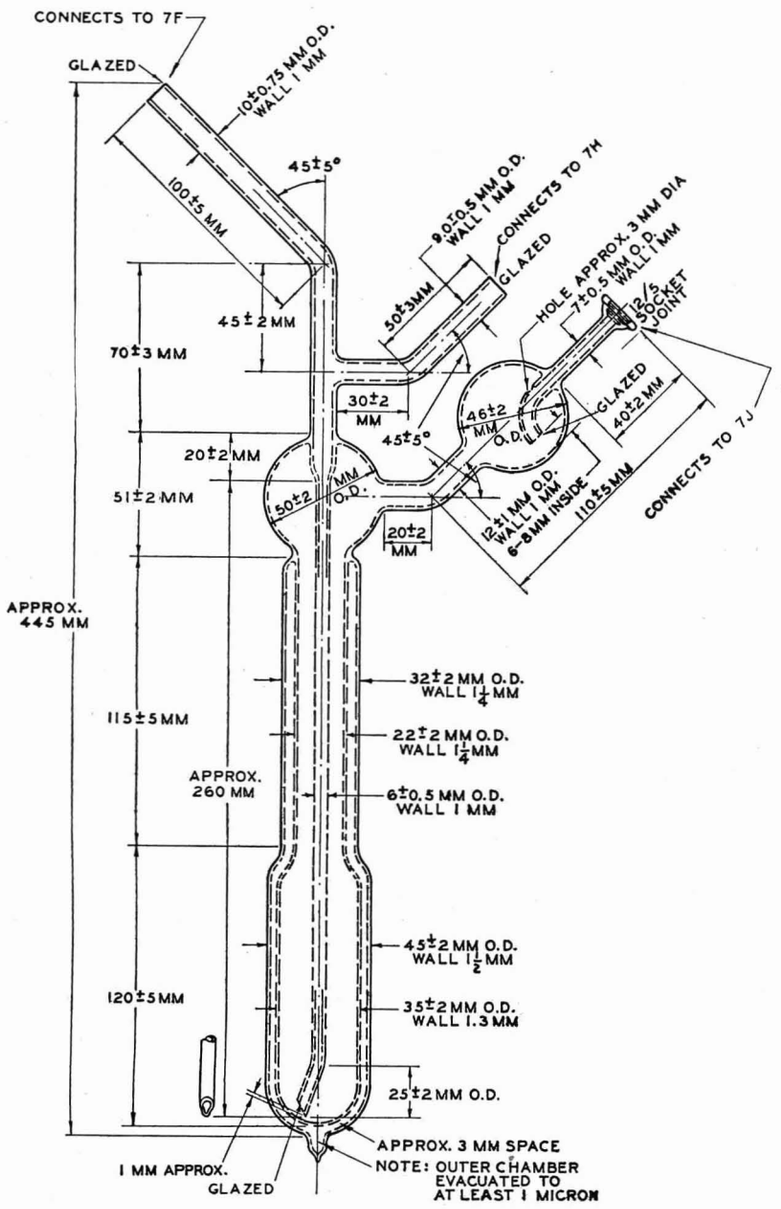


Figure 7G. Distillation Flask

This steam generator is also used with the Kjeldahl distillation apparatus, one-piece model (Figures 8 and 9), described below.

Changes have been made in the conventional designs of the distillation flask, Figure 7*G*, and the condenser tube, Figure 7 *J*. They include a ball and socket joint connection, which should be lubricated with stopcock grease and held together with a suitable clamp, and a West-type condenser.

Kjeldahl Distillation Apparatus. ONE-PIECE MODEL (Figures 8 and 9). An apparatus of this type, but having slightly different dimensions, has been commercially available for some time (Scientific Glass Apparatus Co., Inc., Bloomfield, N. J.). The origin of this apparatus is unknown, although attempts have been made to establish it. Because excellent results have been obtained with a one-piece model by many, including members of the committee, the design was studied. The specifications recommended here are the result of this work.

This revised model is used with the electric steam generator, Figure 7*A*, forming a sturdy, compact unit. The complete apparatus is shown in Figures 8 and 9.

The inner portion of this apparatus is heated by steam flowing through the system, in contrast to maintenance of the temperature by a vacuum jacket as in Figure 7*G*. The steam enters through the vertical tube extending almost halfway up the outer jacket at the left, surrounds the distillation flask proper (inner jacket), and passes into the small bent tube near the top at the right (above the sample funnel inlet), and then downward through the bent portion of the tube at the lower end of the inner jacket, and up into the center portion or the distillation flask. The two traps with T-shaped tubes hold back alkali spray.

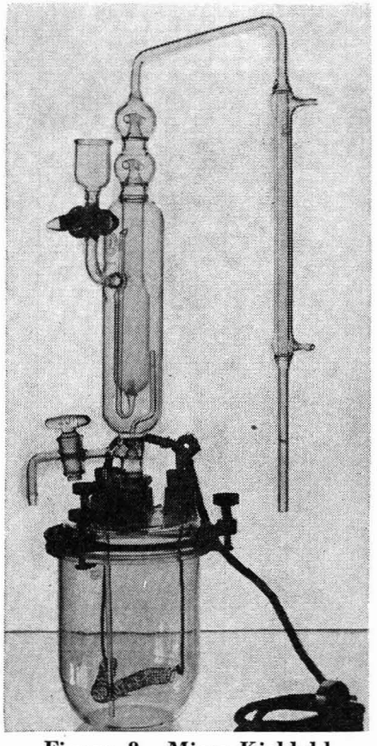


Figure 8. Micro-Kjeldahl Distillation Apparatus

One-piece model

OPERATION. With both stopcocks open, the sample is introduced through the funnel and the curved tube into the inner chamber or distillation flask. The alkali is added in like manner, displacing the acid portion upward. Steam is generated with the stopcocks on both the sample inlet funnel and the drainage tube closed, and the distillation is allowed to proceed.

Several methods of cleaning can be used. The following one has been found satisfactory, particularly when boiling chips are employed in the digestion:

While steam is being generated and with both stopcocks closed, the sample inlet funnel is filled with water and the tip of the condenser immersed in about 100 ml. of water contained in a beaker. Steam generation is stopped, and stopcock of the funnel is opened slowly, being closed before all the water has drained into the apparatus. Reduced pressure causes water to be sucked from the beaker into the apparatus, washing it. The stopcock in the drainage tube is opened to empty the liquid which has collected in the outer jacket, the steam generation is started again, and the procedure is repeated.

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RECEIVED November 10, 1950.

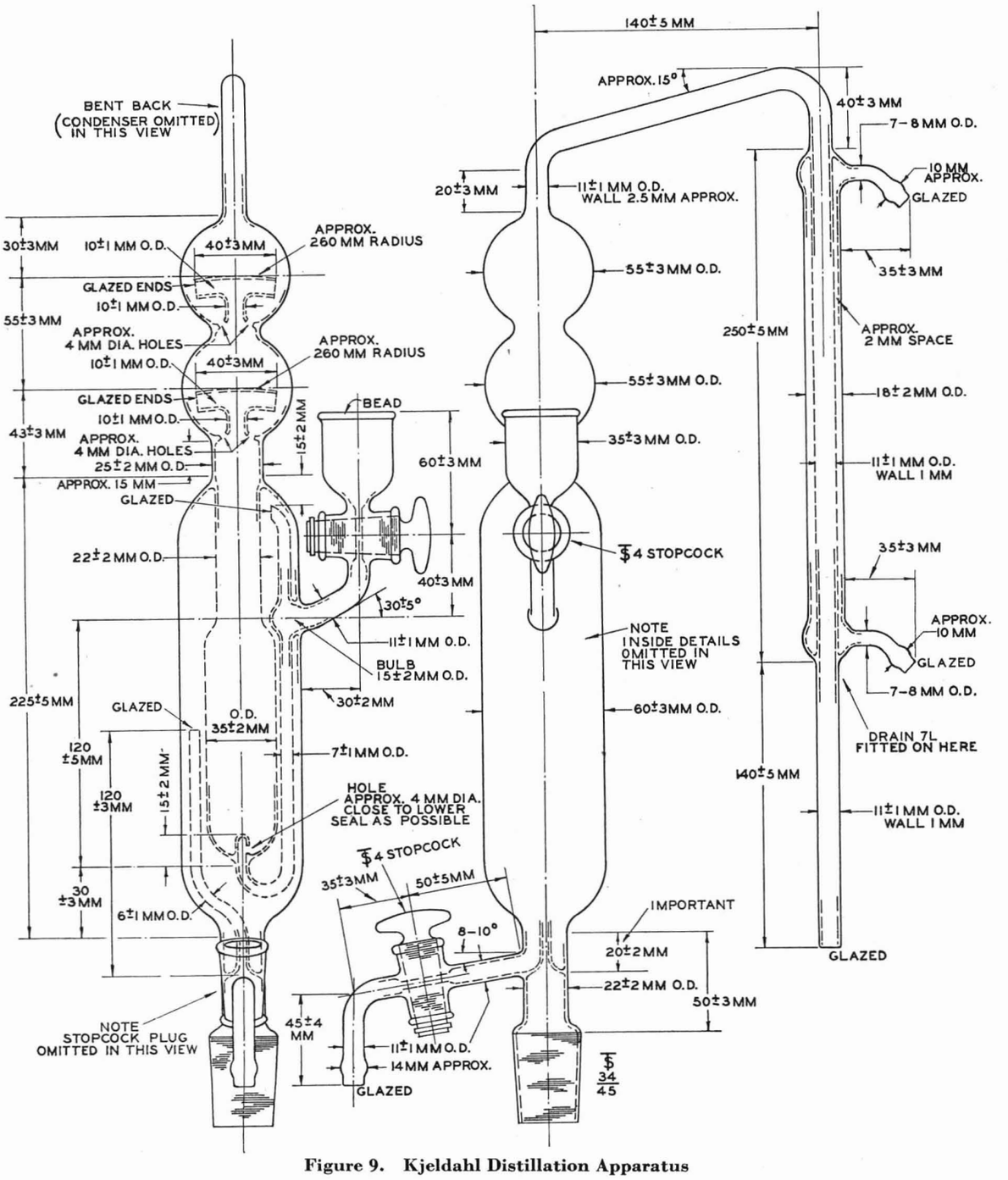

Figure 9. Kjeldahl Distillation Apparatus

One-piece model

Round-Table Discussion

ANALYTICAL DISTILLATION

Digest of stenographic report of round-table discussion held by Division of Analytical Chemistry, 118th Meeting, ACS, Chicago, Ill., September 1950

Moderator: ARTHUR ROSE, *The Pennsylvania State College, State College, Pa.*

THE discussion dealt in succession with concentric-tube columns, effect of nonadiabatic operation, total-reflux distillation, test mixtures, interpretation of distillation curves, spiral-screen columns, and spinning-tube columns. These specific items introduced the more general subjects of apparatus, procedure, testing, and interpretation.

CONCENTRIC-TUBE COLUMNS

R. M. Kennedy of Sun Oil described the small concentric-tube columns (*4*) which are used in the laboratories of his company. These are used for analysis of complex hydrocarbon mixtures, using 10-ml. samples and analyzing the fractions by infrared procedures. The small sample size saves some time, but the big saving is in the scale on which over-all research programs can be executed. The annulus of the columns is about 0.7 mm. and the H.E.T.P. (height equivalent to a theoretical plate) is about 5 mm. at total reflux and 60 ml. per hour boilup rate. The holdup is 0.02 to 0.03 ml. per theoretical plate. The efficiency factor is 2500 reciprocal hours which is exceeded only by certain Podbielniak columns and by the Rossini rotary concentric-tube columns. The rate of product removal is 0.3 ml. per hour, and reflux ratios of up to 200 are used.

It was also brought out that vacuum operation would probably be difficult with the 0.7-mm. annuli, but that for this purpose larger annuli (up to 2 or 3 mm.) had been used successfully by Kuhn. Foster of Standard Oil of Indiana reported difficulty in maintaining boilup rate in vacuum distillation with these columns. He attributed this to large pressure drop caused by too small annular spaces. There was some doubt as to whether the concentric-tube columns would be highly advantageous for distillations requiring a small number of plates and low reflux. Kennedy felt that the rate of approach to equilibrium was probably slow in concentric-tube columns. Schoenberger of Podbielniak, Inc., indicated that the advantageous characteristics of concentric-tube columns had been definitely confirmed.

NONADIABATIC OPERATION

John R. Bowman of Mellon Institute inquired if it was critical to maintain the concentric-tube column in an adiabatic condition. The answer was negative. The columns are fitted with vacuum jackets and operated up to 125° C. without external heaters, and up to 250° C. with a suitable heating mantle. Kennedy stated that data indicated better operation when columns were operated nonadiabatically. Weitkamp of Standard Oil of Indiana reported the same experience with other types of columns. Bowman said this was probably due to occurrence of thermal rectification (*3*). The combined effect of thermal and contact rectification was shown to give improved separation under nonadiabatic conditions. There was agreement that it was difficult to secure adiabaticity and to determine its existence with certainty. C. R. Begeman of Podbielniak, Inc., cited data at total reflux showing increase of H.E.T.P. with increasing heat loss.

METAL SCREEN COLUMNS

J. E. Hawkins of the University of Florida pointed out the desirable characteristics of Lecky and Ewell (*5*) spiral-screen columns for vacuum distillation of relatively viscous liquids. One column with 46 inches of packing, with the spiral screen in the annular space between 18- and 37-mm. tubes, had an H.E.T.P. of less than 1 inch, a holdup of 1 ml. per plate, a total pressure drop of about 2 mm. of mercury when operating at 20 mm., and a head reflux of 2.5 ml. per minute. With smaller columns, H.E.T.P. was 0.6 inch, holdup was 0.15 ml. per plate, and pressure drop was about 2 mm. of mercury. These columns have the same H.E.T.P. at 20 mm. of mercury pressure as at atmospheric pressure.

Hawkins also recommended the use of 5-mm. diameter, twisted-screen packing (*1*) for analytical distillations of small quantities of material.

SPINNING-TUBE COLUMNS

F. D. Rossini of the Carnegie Institute of Technology described briefly the rotary concentric-tube distilling column (*9*) and its characteristics. He stated that a larger model of this apparatus was in process of development. The need for maintaining turbulence in the fractionating zone was pointed out; a rotor 5 inches × 5 feet with an annular space of 1 mm., and operating at 4000 and 8000 revolutions per minute is necessary. At the higher speed, and at a throughput of 1 gallon (liquid) per hour, it is estimated that this apparatus will have a separating power equivalent to 500 theoretical plates.

TOTAL REFLUX DISTILLATION

Rossini brought up the possibility of an analytical distillation in which all of the charge is placed in the rectifying section and brought to equilibrium at total reflux; then, using infrared examination or some other suitable means at various points along the length of the column, the composition could be determined. Rossini described the process as it occurs in any fractionating column as distillation or adsorption; he also described how the number of theoretical stages in a column might be calculated for such a case. H. R. Kaiser of Podbielniak, Inc., indicated that an automatic distillation apparatus operating on this principle was in an advanced stage of development. It was indicated that the time required to reach equilibrium was about the same as in ordinary batch distillation. Begeman stated that experiments had been made in a 3-foot column with temperature measurements every inch to obtain a curve for a six-component hydrocarbon mixture. This was used to calculate a value for total plates in the column that was in excellent agreement with the value obtained by conventional means. There are some unexplained discrepancies in the compositions calculated from such curves, and it is believed these have to do with holdup distribution. Rossini indicated the need for a better method of determining the composition than measurement of the temperature.

COLUMN TEST MIXTURES

R. F. Marschner of Standard Oil of Indiana pointed out the unsatisfactory situation with regard to rapid testing of columns with 100 or more theoretical plates. The mixtures used have too large and too variable a relative volatility, or the methods of analysis are cumbersome. Marschner suggested the use of iso-

topic mixtures as test liquids, and indicated that preliminary experiments had been successful.

Hawkins suggested a mixture of ethylbenzene and chlorobenzene as an ideal test mixture for columns with up to 80 plates, and also for tests at both atmospheric and subatmospheric pressures. Its relative volatility ranges from 1.10 at 760 mm. to about 1.12 at 20 mm. of mercury pressure. The use of an *n*-heptane–methylcyclohexane mixture at reduced pressures was also suggested.

F. E. Williams of Hercules Powder Co. pointed out the lack of test mixtures composed of compounds other than hydrocarbons. Rossini felt this was because hydrocarbons were more easily obtained in the pure state than other types of compounds.

INTERPRETATION OF DATA

Williams pointed out the complexities of analyzing break cuts to obtain over-all composition, and the desirability of being able to calculate composition directly from the boiling point or refractive index curves. Schoenberger recommended the use of the equal-area cut-point method for low temperature distillations, but also indicated this method had definite limitations. Rossini observed that from accurate boiling points and vapor pressure–temperature curves it is possible to calculate the boiling point of an equimolar mixture and thus establish the cut point. The additional difficulties which result from the presence of a third component during the break were noted.

Hawkins described the calibration of columns with known mixtures, in order to obtain arbitrary correction factors for locating cut points and account quantitatively for the material subjected to analytical distillation.

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RECEIVED November 4, 1950.

Round-Table Discussion

Direct Determination of Oxygen in Organic Compounds

Thermal Decomposition Method

Report of round-table discussion held by Division of Analytical Chemistry, 118th Meeting, ACS, Chicago, Ill., September 1950

Moderator: V. A. ALUISE, *Experiment Station, Hercules Powder Co., Wilmington, Del.*
Panel: H. K. ALBER, *Arthur H. Thomas Co., Philadelphia, Pa.*
H. S. CONWAY, *Research Department, Standard Oil Co. (Indiana), Whiting, Ind.*
C. C. HARRIS, *Polychemicals Department, E. I. du Pont de Nemours & Co., Wilmington, Del.*
W. H. JONES, *Esso Laboratories, Esso Standard Oil Co. (Louisiana), Baton Rouge, La.*
W. H. SMITH, *National Bureau of Standards, Washington, D. C.*

ALTHOUGH numerous articles have been published on the direct determination of oxygen in organic compounds, up until the last decade there was no generally used method for this determination. Hans Meyer (*26*) in 1938 advanced the following reasons for this: "The methods...for the determination of oxygen are extremely bothersome and not of general applicability, so that they are scarcely ever used by anyone besides their discoverers."

Walker and Patrick (*35*) were the first investigators to apply the principle upon which the thermal decomposition method is based. They determined oxygen in steel by melting the sample with graphite and measuring the resulting carbon monoxide with iodine pentoxide. Schütze (*29*) used this principle to determine oxygen in zinc dust; at the same time (*30*), he published a general semimicromethod for determining oxygen in organic compounds. This is a thermal decomposition method based on the water-gas reactions.

Shortly after Zimmermann (*38*) had adapted Schütze's method to a micro scale, Unterzaucher (*33*) made improvements in the microprocedure which eliminated any blank correction. He presented an extensive table of remarkably accurate oxygen results on both organic and inorganic compounds.

The excellent review article by Elving and Ligett (*11*) and the work of Aluise *et al.* (*4*) aroused great interest in the Unterzaucher method in this country. Various other investigators (*5*, *7–9*, *15*, *20*, *24*, *36*) have reported this method as satisfactory, and an increasing number of laboratories in this country are now using it routinely.

Essentially, the procedure involves pyrolysis of the sample in a stream of an inert gas and conversion of all the oxygen in the pyrolysis products to carbon monoxide over carbon at about 1120° C., according to the water-gas reactions. The carbon monoxide, which is a measure of the oxygen in the sample, may then be determined in any one of several ways: by oxidation with iodine pentoxide and measurement of the products volumetrically or gravimetrically; by colorimetric measurement; and by thermal conductivity measurement.

OXIDATION METHOD

After oxidation of the carbon monoxide with iodine pentoxide, Schütze (*29*, *30*) and subsequent investigators (*5*, *20*, *38*) weighed the carbon dioxide formed. Unterzaucher (*33*) and other workers (*4*, *7*, *9*, *19*, *24*) determined the liberated iodine titrimetrically after oxidation to iodate. Korshun (*20*) weighed both the carbon dioxide formed and the iodine liberated, and thus claimed to avoid the necessity of doing duplicate determinations. Deinum and Schouten (*8*) reported a procedure in which the carbon monoxide

is oxidized with red mercuric oxide and the resulting carbon dioxide is determined titrimetrically.

Holowchak (*16*) described a modification in which the carbon monoxide is oxidized with copper oxide at 300° C. After removal of water in a dry ice trap, the carbon dioxide in the gaseous pyrolysis products is trapped by liquid nitrogen and determined manometrically in a two-way system, one for small amounts and one for large amounts of carbon dioxide.

In discussing the importance of the direct determination of oxygen in the petroleum industry, Conway (*6*) presented data showing a relative error of 1 to 2% on samples containing over 10% oxygen and a relative error of 5% on samples with lower oxygen content. He stressed that experience and painstaking manipulations are essential to obtain consistent precision and accuracy with the Unterzaucher procedure.

Jones (*18*) discussed the results of the American Petroleum Institute cooperative program for oxygen analysis being conducted by his A.P.I. subcommittee on analysis of oxygenated compounds. In discussing the Unterzaucher procedure, he stated the following:

1. Although the participating laboratories reported excellent precision on several synthetic mixtures, the accuracy varied and considerable difficulty was experienced in obtaining satisfactorily low blanks.
2. Based on experiences reported to date, the analysis by this procedure appears to be somewhat of an art, and only after repeated and continuous efforts can any laboratory, trying for the first time, obtain even partially satisfactory results.
3. Further research on this method is necessary in order to eliminate some of the uncertainties which are apparently inherent in the method.

Alber (*1*) discussed the latest developments in the method by European chemists. He reported that, from his many discussions with investigators abroad, it appears that the Unterzaucher procedure is most frequently used for determination of oxygen.

Those laboratories using the oxidation method for determining the carbon monoxide have reported satisfactory results for oxygen in a variety of materials, such as resins, aliphatic and aromatic compounds, terpene derivatives, oxygen-containing polymers, coal, bituminous and petroleum products, and various inorganic substances. The amount of oxygen in these materials ranged from a few hundredths per cent to approximately 76%. In many instances, nitrogen, sulfur, and halogens were also present, alone or in combination. Most workers did not encounter any difficulty with compounds containing any of these constituents. However, Maylott and Lewis (*24*) reported high results for oxygen on compounds containing sulfur, as a result of the formation of hydrogen sulfide, carbon disulfide, and carbonyl sulfide; the presence of these compounds was established by the mass spectrometer. Maylott and Lewis found that carbon disulfide and carbonyl sulfide, both of which liberate iodine from iodine pentoxide, are not removed by Ascarite and, therefore, they recommend the use of a liquid nitrogen trap for this purpose.

Unterzaucher (*34*) found that substances containing phosphorus or fluorine give high values and also have a harmful effect on the combustion tube filling so that any subsequent determinations are unsatisfactory. On the other hand, Walton *et al.* (*36*), who determined the carbon monoxide by direct colorimetric measurement, did not experience any difficulty with phosphorus-containing compounds.

Kuck (*21*) reported that he was able to obtain satisfactory results on compounds such as acetanilide, but not on compounds such as benzoic acid. He attributed this to not having a sufficient length of carbon filling at 1120° C. to effect complete reduction of the carbon dioxide formed by decarboxylation of the benzoic acid. Harris (*14*) pointed out that the rate of conversion of carbon dioxide is not the same as for water. According to Harris *et al.* (*15*), the rate of the steam-carbon reaction is retarded by adsorbed hydrogen, whereas the rate of the carbon dioxide–carbon reaction is reduced by both hydrogen and carbon monoxide.

Huffman (*17*) experimented with iodine pentoxide at temperatures from 25° to 125° C. A blank was not obtained at any temperature in this range. The oxygen recovery, on all samples analyzed, was low (95 to 96%) at room temperature and extended sweeping was required to carry all of the iodine into the absorption tube. At 125° C. satisfactory results were obtained on some compounds (acetanilide) and high results were obtained on others (cholesterol). All samples analyzed gave consistently satisfactory results at 70° to 80° C. He attributed the high results at 125° C. on certain compounds to reactions of organic gaseous products with the iodine pentoxide. Huffman also found that various samples of iodine pentoxide had different activities.

COLORIMETRIC METHOD

A method for determining small amounts of oxygen in rubbers, plastics, petroleum products, and other organic materials by direct colorimetric measurement of the carbon monoxide was discussed by Smith (*32, 36*). In this method, interfering substances are removed from the products of pyrolysis by either a liquid air or potassium hydroxide trap before collection of the gas. The percentage of carbon monoxide in the collected gas is determined by means of a National Bureau of Standards colorimetric indicating gel (*31*). The method has been used successfully on organic materials containing from 0.01 to 6.3% oxygen.

THERMAL CONDUCTIVITY METHOD

Harris (*14*) described a modified method (*15*) for the determination of oxygen by pyrolysis of the sample in a known volume of helium at a reduced pressure, and circulation of the pyrolysis products over carbon at 1100° C. In this procedure, all but the most stable carbon oxide complexes are removed from the carbon by a pre-evacuation. During circulation hydrogen is removed selectively by diffusion through a heated palladium tube, and the rate of carbon monoxide formation is followed by recording the change in thermal conductivity of the gas mixture. When the conversion to carbon monoxide and removal of hydrogen are complete, as shown by attainment of constant potential, the composition of the helium–carbon monoxide binary mixture can then be obtained from this potential and a calibration curve. The volume of helium in the binary being known, the amount of oxygen in the sample is readily calculated.

APPARATUS AND REAGENTS

Furnace. The unusual high temperature (1120° C.) is a severe requirement and is not fulfilled by the conventional combustion furnace. Some investigators have constructed their own furnaces using platinum wire heating elements. Others (*9, 24*) have used a Hoskins Type 303A or an Arthur H. Thomas No. 5674 furnace, both of which utilize base metal heating elements and are available commercially. In order to provide a sufficiently high temperature in that portion of the tube passing through the insulation at the end of the furnace, Aluise (*2*) has designed a ring gas burner for use as an auxiliary source of heat.

Reaction Tube. The reaction tube is usually made of transparent quartz. Devitrification of the quartz can be retarded by maintaining the tube at 700° to 800° C. when not in use. When devitrification becomes severe, the affected portion of the tube can be cut out and replaced by a new section. Aluise (*3*) found that Solar Radiation grade quartz, Hanovia Chemical and Manufacturing Co., has a longer life than ordinary commercial grade quartz.

Carbon. Two important requirements of this material are an amorphous structure and a low ash content. It may be prepared from benzene or acetylene (*4, 5, 8*). Unterzaucher, who used a gas black carbon, purified it by treatment with hot dilute hydrochloric acid. Wyex Compact Black, a pelleted carbon available from the Arthur H. Thomas Co., has been used by several investigators, both as received or after treatment with hot dilute hydrochloric acid (*3, 4, 9, 15, 24*). Before use, it is necessary to heat the carbon, preferably overnight, at 800° to 900° C. This sinters the carbon and thereby avoids channeling during use in the reaction tube. Pyrex wool should never be used to hold the carbon in place during this treatment; contamination of the carbon by borosilicate glass wool will cause high and erratic blanks. Quartz wool or platinum gauze is recommended for this purpose.

The length of the carbon filling is usually 12 to 15 cm.; no investigator has shown that a longer carbon filling is necessary. According to Elving (*10*) a catalyst consisting of carbon and copper appears to have promise for conversion of oxygen to carbon monoxide at lower temperatures.

Nitrogen. The nitrogen must be essentially free from oxygen or oxygen-containing constituents, in order to obtain a satisfactorily low blank. Both Linde high purity, dry nitrogen and Air Reduction Seaford grade nitrogen have been found satisfactory. The latter grade contains approximately 1% hydrogen. According to the manufacturers' specifications, neither grade contains more than a few thousandths of 1% of oxygen.

As a precaution, most workers pass the nitrogen through a purification system. Reduced copper at 400° to 500° C. is most commonly used for this purpose, although other reagents have been reported (*5, 8, 20*).

Helium. Helium has been used by some workers (*15, 36*). Traces of oxygen are removed with reduced copper at 400° C.

Iodine Pentoxide. Three commercial brands found satisfactory are Baker's C.P., Eimer and Amend, and Merck's. According to Alber (*1*), Unterzaucher recrystallized iodine pentoxide from concentrated nitric acid to exclude water-insoluble constituents. Huffman (*17*) prepared this reagent in a granulated form by dehydration of iodic acid. According to Lamb *et al.* (*22*), iodic acid may be prepared by oxidation of iodine with either nitric or chloric acids. However, Welton and Drake (*37*) emphasized that the iodine pentoxide obtained by dehydration of iodic acid gave low and consistent blanks only when the latter had been prepared by the chloric acid method.

Some workers condition the iodine pentoxide by heating it 3 to 8 hours in vacuum at 180° C., whereas others heat it in a stream of dry nitrogen for 24 hours or more at 230° to 240° C., followed by 40 to 50 hours at 150° to 160° C. According to Mellor (*25*), if the nitrogen is inadequately dried, or if the drying temperature exceeds 250° C., the iodine pentoxide may become brown, probably because of the liberation of iodine.

BLANK VALUE

Considerable time was devoted to discussion of the difficulties encountered by various workers in obtaining consistently low blanks. Schütze (*30*) reported that the blank was negligible in his semimicro method. Zimmermann (*38*) obtained a constant blank of 0.100 mg. of carbon dioxide (equivalent to 0.27 ml. of 0.02 *N* sodium thiosulfate) in his micro adaptation of Schütze's method. Unterzaucher's (*33*) improvements in the micromethod eliminated this blank. Huffman (*17*) reported that he obtained no blank with Unterzaucher's procedure. Holowchak (*16*) reported that although he obtained a blank on a fresh filling of carbon, after several weeks this blank would disappear. Elimination of the blank by use of the thermal conductivity technique was reported by Harris *et al.* (*14, 15*).

Aluise (*3*) found that digestion of Wyex Compact Black with dilute hydrochloric acid and the use of Linde high-purity, dry nitrogen reduced the blank from approximately 0.5 ml. to a consistent value in the order of 0.05 ml. of 0.02 *N* sodium thiosulfate. According to Alber (*1*), Unterzaucher insists that a blank should not be tolerated even if it is consistently reproducible. However, other workers have reported favorable results with the thermal decomposition method using rather large blanks, equivalent in some cases to as much as 4 ml. of 0.02 *N* sodium thiosulfate in a total titration of 33 ml.

Various theories were advanced to explain the high blanks. Alber (*1*) reported that the first blank was obtained in Unterzaucher's laboratory 3 years after introduction of the method. After investigation, the blank was traced to several of the following sources: (*a*) hydrogen and traces of oxygen in the nitrogen, both of which should be removed in the purification system; (*b*) variations exceeding ±5° C. in the cracking furnace temperature (1120° C.); (*c*) improper cleaning of the quartz reaction tube (40% hydrogen fluoride is recommended instead of cleaning solution to remove surface impurities); (*d*) failure to use ash-free carbon; and (*e*) impurities in the iodine pentoxide not removed by recrystallization from water. Unterzaucher also recommends that paraffin oil be used in the bubble counter instead of sulfuric acid. The former is said to be adequate for about 500 determinations.

The reduction of the quartz to silicon monoxide by the carbon at high temperatures has also been advanced as a possible reason for the high blank which is often encountered (*3, 7, 8, 13, 19, 23, 27*). Aluise (*3*) found that raising the temperature from 1120° to 1350° C. doubled, and sometimes tripled, the blank with a badly devitrified quartz tube but had only little effect on the blank with a new quartz tube.

Another reason for high and inconsistent blanks was advanced by Orning (*28*). He found that a carbon of the type used in the thermal decomposition method can store the equivalent of approximately 0.5 ml. of carbon monoxide (0.36 mg. of oxygen) at about 700° C. He stated that if the temperature of the carbon near the ends of the furnace is 800° or 900° C., it can strip oxygen from carbon dioxide and release this oxygen later under varying conditions, such as an increase in temperature or a change in the composition of the gaseous products passing over it. For this reason, he advised that all of the carbon be maintained at the same temperature (1120° C.). In support of Orning's findings regarding the retention of carbon monoxide by carbon, Harris (*14*) cited the work of Emmett (*12*). He also suggested the evacuation of the carbon, in the Unterzaucher procedure, as a possible means of lowering the blank.

SUMMARY

Most investigators have reported satisfactory results with the thermal decomposition method for the determination of oxygen, but they have emphasized that the necessary conditions for carrying out the analysis must be carefully observed. Virtually the only modifications which have been made in the Schütze or Unterzaucher procedure are: the circulation of the pyrolysis products, the removal of hydrogen, and the method of determining the carbon monoxide formed. In general, the work reported to date definitely indicates that the thermal decomposition method is basically sound and is proving to be of increasing usefulness in ultimate analysis. The additional information presented at this round-table discussion should prove to be of great value to the chemist now using the method as well as to the chemist who plans to set it up for the first time.

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NOTES ON ANALYTICAL PROCEDURES . . .

Apparatus for Microdetermination of Molecular Weights by the Vapor Density Method

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THE method of determining molecular weights described by Niederl and Niederl (*1*) proved accurate for structural work done in the authors' laboratory on low boiling substances. For high boiling liquids, however, they found it necessary to design their own apparatus, because no commercial equipment of this type was available. The apparatus developed works equally well for liquids of high and low boiling point and has the added advantage that the techniques involved in its use are much simpler than those required for other such apparatus. A determination—weighing the sample, assembling and filling the apparatus, and making the run itself—requires only 10 minutes.

APPARATUS AND PROCEDURE

The principle is the same as that used by Niederl—namely, the determination of the volume of vapor obtained from a weighed sample of the unknown, by means of the weight of mercury it displaces. The apparatus (see illustration) consists essentially of three parts: tube *G*, the constant-temperature bath; the vaporizer, *A*, *B*, *C*, *D*; and the mercury dispenser, *F*, which is used in filling the vaporizer with mercury.

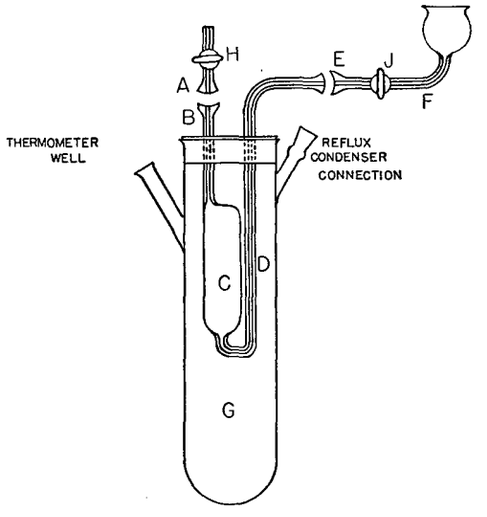
G is constructed from a 38 × 300 mm. borosilicate glass test tube by sealing on one side tube to admit a thermometer and a second side tube for use in attaching a reflux condenser. A three-necked round-bottomed flask has been used with equal success.

Dispenser *F* is constructed from a borosilicate glass thistle tube fused to a stopcock, *J*, to the other side of which is attached a 12/2 standard socket of 2-mm. capillary bore. It is essential that this last be no larger in diameter, if mercury spillage is to be avoided.

The vaporizer tube, *C*, is made from a 15 × 125 mm. borosilicate glass test tube. The flattening of its top and the offset position of inlet tube *B* are essential to prevent the lifting of the sample tube into *B* and the trapping of air in *C* when the apparatus is being filled with mercury.

Tube *D* is a capillary of not more than 2-mm. bore; *B* is 100 mm. long and must be as near 2-mm. bore as possible, in order just to admit the sample tube. The bore must be no larger, as the mercury retained within it during the vaporization of the sample prevents vapor from reaching the ball joint, *A*. The top of *D* must be a little higher than *A;* otherwise, when the mercury dispenser is removed after the apparatus has been filled with mercury, air may leak through *A* and allow the mercury level in *B* to fall.

The preparation of the mercury and the weighing of the sample in a capillary tube container small enough to pass through *B* are essentially as described by Niederl. The sample bulblets are made of ordinary melting point capillary. One end is sealed and drawn to a slender rod about 3 cm. long to serve as handle; the other end is drawn to a fine capillary 4 to 5 cm. long, the bulb itself being 1 to 3 cm. long. The sample is introduced into the weighed container by warming the bulb, dipping the capillary tip into the liquid, and cooling to draw liquid into the bulb. The bulb is set upright, shaken or centrifuged, then reweighed. Extremely volatile liquids are sealed in by touching a flame momentarily to the capillary tip, which is broken off just before the bulb is placed in the apparatus. The sample tube is then dropped through *B*, and joint *A* is assembled and clamped with the usual spring clamp. The stopcock, *H*, which is lubricated sparingly (preferably with Dow-Corning silicone grease), remains open during the filling operation. The mercury dispenser, *F*, is next filled and cleared of all air by flushing mercury through it into a clean beaker. The dispenser is then attached to the vaporizer unit at joint *E*. Stopcock *J* is opened slowly and mercury is allowed to flow slowly into the vaporizer unit.



If joint *E* has not been greased adequately, air will be sucked into the tube intermittently, making the filling difficult; however, if this condition is detected early enough, the filling can proceed normally after the joint has been regreased. As the mercury rises in *C* the weighing capillary usually moves over to the side wall and adheres there, but occasionally it may be necessary to ensure its entrapment at the flattened top by tilting the apparatus slightly. When the mercury has risen past stopcock *H*, the latter is closed. Stopcock *J* is then closed, the dispenser is disconnected, and a weighed vial is placed at *E* to collect the expelled mercury.

Table I. Compounds Suitable for Vapor Bath

Compounds	B.P., ° C.
Water	100
Toluene	110
Isoamyl acetate	142
p-Dichlorobenzene	174
Ethylene glycol	197
Methyl salicylate	223
Isoamyl benzoate	262
Diphenylamine	302
Benzyl benzoate	323
n-Butyl phthalate	340

Table II. Performance Data

Sample	B.P., ° C.	Molecular Weight	
		Theoretical	Experimental
Acetone	56.5	58	58
Benzene	80.1	78	79
Toluene	110.8	92	95
Chlorobenzene	132.0	113	114
Ethyl benzoate	212.6	150	153
Aniline	184.4	93	91

After the apparatus is assembled and checked, a liquid of suitable boiling point (see Table I) is placed in *G* and heated to boiling. This boiling is continued until no more mercury is expelled. If the temperature tends to climb slowly, a final temperature may be arbitrarily chosen, provided the flame is removed immediately after the reading is taken. When the final temperature is noted, the height of the vertical column of mercury as measured from the meniscus in the vaporizer to the level of the orifice of the capillary outlet at *E* is also recorded.

The usual corrections for the expansion of the mercury and the air error are made by means of a blank run, using an empty weighing capillary. It has been the practice to run a blank for each bath liquid used. The average correction per degree change in temperature can be calculated from one such run and applied in the way described by Niederl. Other corrections also are handled as in Niederl's method.

After completion of the run, the apparatus may be quickly cleaned out and made ready for the next run by the following procedure. A suction-flask assembly that connects with ball joint *E* via a socket joint is clamped into place. Suction is applied, stopcock *H* is opened, and the mercury in the vaporizer unit is pulled into the suction flask. The constant-temperature bath is operated during this process so that the sample remains vaporized and may be evacuated as the mercury is being drawn off. By successively opening and closing stopcock *H*, it is possible to flush the vapors of the sample from the vaporizer quantitatively. If need be, a solvent can be flushed through the vaporizer by introduction through joint *A*. The old weighing capillary may then be removed and the apparatus is ready for the next run.

RESULTS

The excellent performance of this apparatus is illustrated by the results in Table II, which were obtained by a senior chemistry student who had had no previous experience with this apparatus.

These results were obtained on samples of 10 to 15 mg. weighed on a good analytical balance. Although the use of a microbalance might have given closer results in some cases, those given here are typical of the many determinations run. The capacity of the apparatus described is such that larger samples might readily be used. With water as the liquid used in the vapor bath, an 11-mg. sample of acetone displaced only one third of the available capacity.

Because the primary utility of such an apparatus as this is in the straightforward and rapid determination of molecular weights, the vapor bath has been employed throughout. A liquid bath can be easily substituted, if the boiling and condensation points of a liquid are desired.

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RECEIVED April 10, 1950.

Quantitative Determination of Bromine in Terminal Bromodinitromethyl Groups

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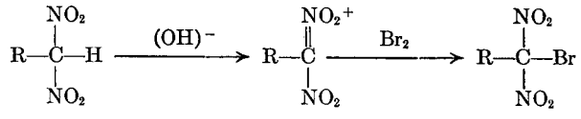
THE study of compounds with terminal dinitromethyl groups made it desirable to find a facile laboratory method for their characterization. Compounds with terminal dinitromethyl groups are readily brominated in basic solution to form the corresponding bromodinitro derivative. Because the bromodinitro derivatives are easily prepared and purified, halogen analysis of these compounds seemed to offer a convenient method for the quantitative determination of terminal dinitromethyl groups.

Table I. Determination of Bromine

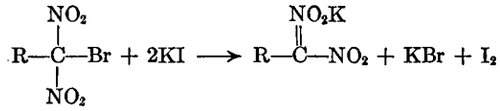
	Compound	%Br Calcd.	%Br Found	% Dinitromethyl Group Calcd.	% Dinitromethyl Group Found
1	$Br-C(NO_2)_2-CH_2CH_2COOCH_3$	29.49	29.58 29.92	38.75	38.88 39.33
	$Br-C(NO_2)_2-CH_2CH_2COOH$	31.10	31.06 31.29	40.87	40.83 41.12
3	$Br-C(NO_2)_2-CH_2OH$	37.17	36.85	48.86	48.43
4	$Br-C(NO_2)_2-Br$	60.57	60.19 59.89	...	...
5	$CH_3-C(NO_2)_2-Br$	40.16	40.11 40.08	52.79	52.72 52.68



Two methods of halogen analysis appeared worthy of consideration: treatment of the compound with an alkaline reagent, followed by titration of the ionic halogen with silver nitrate; and treatment of the compound with potassium iodide in the manner reported by Meisenheimer (*1*) for 2-bromo-2,2-dinitro-1-ethoxyethane, followed by titration of the free iodine with sodium thiosulfate.

The first method was found unsatisfactory. In order to obtain the halogen in ionic form, it was necessary to treat the compound with an alkaline reagent in an organic solvent. Colored solutions resulted from this operation, which obscured the end point of the silver nitrate titration.

The reaction of compounds with terminal bromodinitromethyl groups and potassium iodide proceeded quantitatively. For every compound investigated, the amount of iodine formed corresponded to the theoretical amount of bromine present in the molecule according to the following equation:



The percentage of bromine in a compound may be expressed by the formula,

$$\frac{79.92 \times N \times V}{2 \times 10 \times W}$$

where V = milliliters of sodium thiosulfate, N = normality of sodium thiosulfate solution, and W = sample weight in grams.

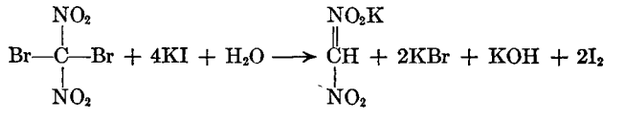
The percentage of terminal dinitromethyl groups can be obtained readily from the same formula by substituting the formula weight of the dinitromethyl group, 105.04, for that of bromine, 79.92.

Table I shows the results obtained with this analytical method.

EXPERIMENTAL

General Procedure. To 0.2 to 0.3 gram of the bromodinitro compound dissolved in 25 ml. of methanol are added about 2 grams of potassium iodide. Free iodine is liberated at once and after a few seconds the mixture is diluted with 25 ml. of water. Then the free iodine is titrated with 0.1 N sodium thiosulfate solution in the usual manner, using starch indicator.

Titration of Dibromodinitromethane and Compounds Having Basic Amino Groups. Upon reaction of dibromodinitromethane with potassium iodide, the solution becomes alkaline according to the following equation:



It is necessary to neutralize the solution with dilute sulfuric acid to a pH of 6 to 7 before titration with sodium thiosulfate. The same is true of the titration of compounds containing substituted amino groups, which cannot yet be tabulated because they are still classified. A better end point is obtained in neutral solution; therefore acidification is necessary only in these two cases.

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RECEIVED June 19, 1950. Work performed under Contract N7onr-1462, Task Order I with the Office of Naval Research.

Analysis by Permanganate Titrations of 8-Quinolinols

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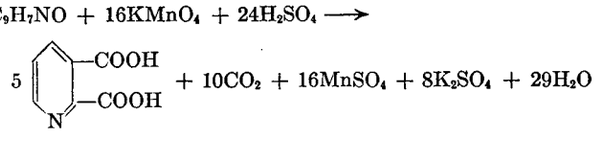
ALTHOUGH a permanganate titration method for determining metals with 8-quinolinol has been known for a long time (*1*), the procedure is stated to be empirical and not sufficiently well investigated to be very useful. Therefore this method has been reinvestigated.

By the modifications specified in the experimental procedure below the reaction can be made to correspond to a feasible equation over a narrow range of conditions if an excess of standard permanganate is added and back-titrated, after some time has been allowed for the reaction to be completed, with iodide and thiosulfate. The equation used for the calculation of the results was as follows:

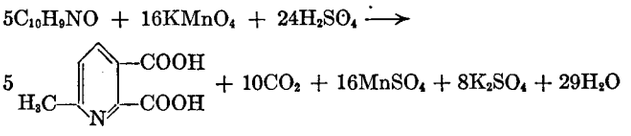


The average error in the determination of seventeen samples of pure 8-quinolinol ranging in size from 13.0 to 29.0 mg. was 0.4 mg.; the length of time allowed for the reaction was 15 minutes and the excess of permanganate about 2 ml. The reaction time and the amount of excess permanganate must be carefully controlled, because the results become progressively higher with longer times and larger excesses than those stated—for example, the results are about 10% high when either the time for the reaction or the excess of permanganate is doubled. Although this indicates that the oxidation proceeds farther than the equation provides for, no limit to the extent of oxidation could be obtained within the range of conditions usable for practical analysis. Evidently the determination is empirical, but is stoichiometric with the equation written when the conditions are controlled as specified.

The procedure was applied to the analysis of seven synthetic copper samples with the results shown in Table I.

Presumably, results of the same order of accuracy would be obtained in the determination of other ions quantitatively precipitated by 8-quinolinol.

Although this method may be satisfactory for many purposes, it was found that the similar titration using 8-hydroxyquinaldine in place of 8-quinolinol was much better, in that the range of conditions over which the reaction obeyed the equation



seemed to be unlimited, in spite of the fact that the products in the equation as written are probably not the true products of the reaction.

Table I. Copper Determination by Permanganate Titration of 8-Quinolinol

% Cu Present	% Found	% Cu Present	% Found
7.38	7.36	4.84	5.07
8.97	9.29	6.90	7.09
8.28	8.59	7.67	7.87
8.67	8.90		

The analysis of seventeen samples of pure 8-hydroxyquinaldine ranging in size from 13.0 to 31.7 mg. gave values correct to 0.3 mg. or better in every trial, even when the quantity of excess permanganate and the length of time allowed for the reaction varied widely. This reaction appears to be truly stoichiometric rather than empirical, in contrast to the similar reaction of 8-quinolinol. The procedure was applied to the analysis of prepared magnesium samples with the results shown in Table II.

EXPERIMENTAL

Reagents. 8-Quinolinol was purified by recrystallization from alcohol; 8-hydroxyquinaldine was prepared and purified as previously described (*2*).

Table II. Magnesium Determination by Permanganate Titration of 8-Hydroxyquinaldine

Mg Taken, Mg.	No. of Runs	Mg Found, Mg.
1.22	2	1.24 ± 0.01
1.52	4	1.52 ± 0.03
1.83	2	1.83 ± 0.01
2.28	2	2.23 ± 0.01
2.44	1	2.42
3.04	2	3.02 ± 0.02

Determination of Copper with 8-Quinolinol. A sample containing about 5 mg. of cupric ion is taken for analysis, or an aliquot portion of a larger sample may be used. After precipitation of the copper in the usual fashion, the precipitate is filtered, washed, and dissolved in approximately 10% sulfuric acid. To this solution is added a volume of 0.1 *N* permanganate amounting to an excess of 0.5 to 2.0 ml. over the theoretical amount. The solution is allowed to stand for 15 minutes, after which 0.5 gram of potassium iodide is added and the liberated iodine is titrated with standard thiosulfate. Accurate results will not be obtained if the excess of permanganate or the time allowed for the reaction differs more than slightly from the values specified; the other conditions of the procedure are not critical.

Determination of Magnesium with 8-Hydroxyquinaldine. A sample containing approximately 0.01 to 0.03 gram of magnesium is precipitated in the usual fashion (*2*) with 8-hydroxyquinaldine. The washed precipitate is dissolved in 10% sulfuric acid and an aliquot portion containing 10 to 30 mg. of 8-hydroxyquinaldine is taken for titration. An excess of from 0.5 to 10 ml. of 0.1 *N* permanganate is added, and the solution is allowed to stand 15 minutes or longer before potassium iodide is added and the iodine liberated is titrated with standard thiosulfate.

CONCLUSIONS

The permanganate titration methods using either 8-quinolinol or 8-hydroxyquinaldine are suitable for the determination of small weights of the metals precipitated by these reagents such as magnesium and copper, inasmuch as the equivalent weight (for divalent metals) is only $^1/_{32}$ of the atomic weight. The results with 8-hydroxyquinaldine are considerably the more satisfactory, owing to the greater ease with which this compound is oxidized to the stoichiometric extent.

ACKNOWLEDGMENT

The authors wish to acknowledge the aid of C. M. Scott on some of the preliminary work for these methods, and the financial assistance of a grant from the Cottrell Research Corp.

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RECEIVED June 22, 1950.

Use of U.S.P. XIV Capsules of Vitamin A Reference Standard

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TWO alternative procedures are permitted by the U. S. Pharmacopeia for the preparation of bioassay feeding solutions from capsules of the U.S.P. vitamin A reference standard (*1*).

"The capsules are so constructed that when an accurate weight of a portion of the contents is desired, it is convenient to cut off the capsule tip and express a portion of the material into a weighing vessel. For use in preparing oil dilutions for biological assay, quantitatively remove the capsule contents by immersing the capsule in a measured volume of vegetable oil, and then cut the capsule with a fine pointed scissors."

Thus, a weighed sample of oil from the capsule may be diluted to an appropriate concentration, or a capsule may be used in toto and assumed to supply 250 mg. (±0.5%) of the reference oil (10,000 U.S.P. units per gram) to the diluent oil in which it is immersed. Use of this second alternative procedure may result in incomplete extraction of oil from the capsule, leading to errors of considerable magnitude in the preparation of oil dilutions for biological assay.

Three analysts were assigned the preparation of a series of vitamin A feeding solutions for bioassay according to both sets of U. S. Pharmacopeia directions.

Solutions of approximately 40 U.S.P. units per gram of the U.S.P. reference standard were prepared in fresh refined cottonseed oil (Wesson). Test solutions obtained by cutting capsules under oil were compared with controls obtained by diluting a weighed amount of reference oil expressed from the capsules. Test solutions were thoroughly mixed following the specified procedure, whereas the control solutions were mixed solely by vigorous swirling. Final solutions were assayed on a Beckman spectrophotometer in cyclohexane or colorimetrically by the Carr-Price reaction, using in both instances a blank containing an appropriate amount of diluent oil.

Table I. Efficiency of Various Capsule Extracting Methods

Method of Mixing	Method of Cutting Capsule	Concn. of Vitamin A, % of Weighed Control
Swirling	In half, crosswise	61.8
Swirling	In half, lengthwise	91.5
Swirling	Many pieces	90.7
Stirring rod	In half, lengthwise	95.7
Rotary paddle	In half, lengthwise	99.7
Rotary paddle	Many pieces	99.5

Scissors and forceps were thoroughly rinsed by the diluent oil. Subsequent rinsing in chloroform yielded a solution which gave no detectable blue color on addition of antimony trichloride. Tumbling in a mixing machine was included under swirling. Mixing procedures were continued for at least 15 minutes and, in most cases, much longer.

The results of various cutting and mixing procedures given in Table I show that it is difficult to extract with oil all the vitamin A from U.S.P. reference standard capsules. Bioassays against such defective standard solutions will yield results correspondingly too high. Rapid movement of the gelatin shell relative to the diluent oil is necessary to remove the film of reference oil from the gelatin. Such agitation can be satisfactorily provided only by using a high-speed rotary paddle stirrer.

It is recommended that the instructions for use of the U.S.P. vitamin A reference standard be modified to require a procedure employing a dilution of a weighed sample of the contents of the U.S.P. standard capsule.

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RECEIVED March 25, 1950. Communication 168, Distillation Products Industries.

Tabulation of Apparatus Used for the Micro-Dumas Determination

Committee for the Standardization of Microchemical Apparatus, Division of Analytical Chemistry, AMERICAN CHEMICAL SOCIETY

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WITH the increase in the number of microchemical laboratories in recent years, and of the individual ideas probably existing in each, the committee felt that there might be a wide variety of microchemical apparatus used. Because no data were available on the extent of such variation, a questionnaire on the apparatus used in the Dumas determination of nitrogen was circulated in 1948, requesting such data as size of sample and description of apparatus parts. It also included a listing of those pieces of apparatus in use by the analyst which conformed to recommended specifications (*2*, *3*). Sixty-four laboratories replied and in many instances reported more than one condition and type of equipment, or failed to reply to specific questions.

The committee believes that the information gained through this questionnaire is of general interest and has prepared the following summary of the data:

1. Sample Technique

53 used 0 to 5 mg.
34 used 6 to 10 mg.
16 used 11 to 50 mg.

26 used mixing tubes
17 were 61 to 80 mm. long
14 were 9 or more mm. in diameter

12 used a platinum boat
6 were 11 to 15 mm. long
9 were 3.6 to 5 mm. in width

43 used porcelain boats
25 were 11 to 20 mm. in length

2. Carbon Dioxide Source

35 used dry ice
13 used marble
7 used calcite
3 used carbon dioxide from cylinders
6 used other sources

3. Carbon Dioxide Generators

KIPP

17 used one Kipp generator
13 were according to specifications (*3*)
3 used two Kipp generators in series
5 had a mercury trap

DEWAR

18 used 1000-ml. capacity or less
10 used 1000 to 2000 ml.
4 used larger than 2000 ml.
20 reported charge lasting from 0 to 10 days
10 reported 11 to 20 days
2 reported longer than 20 days
29 used safety trap
5 did not use safety trap

CARBON DIOXIDE FROM CYLINDERS

3 used this type

4. Gasometer

23 used gasometer
19 were according to specifications (*3*)

5. Combustion Tube and Generator Connection

48 used a Z-tube of Pregl type

6. Combustion Tube

TUBE DIMENSION

46 were according to specifications (*3*)

TUBE COMPOSITION

18 used quartz
17 used Pyrex 172
29 used Vycor

CONNECTION BETWEEN CARBON DIOXIDE SUPPLY TUBE AND COMBUSTION TUBE

50 used a rubber stopper

IS THERE FREQUENCY OF BREAKAGE OF TIP OF COMBUSTION TUBE ON INSERTION?

8 reported yes
48 reported no

SIZE OF RUBBER TUBING BETWEEN EXIT END OF COMBUSTION TUBE AND STOPCOCK OR NEEDLE VALVE

42 used tube of outside diameter 7.1 to 11 mm.
39 used tube of inside diameter 1.1 to 2 mm.
16 used 2.1 to 3 mm. inside diameter

IS SHAPE OF TIP OF RECOMMENDED COMBUSTION TUBE (*3*) SATISFACTORY?

55 answered yes
2 answered no

DIMENSIONS OF CAPILLARY TIP OF COMBUSTION TUBE

Length
15 were 26 to 30 mm.
30 were 31 to 35 mm.

Outside diameter
28 were 2.6 to 3 mm.
10 were 3.1 to 3.5 mm.

7. Stopcock or Needle Valve

TYPE

53 used glass stopcock
40 were according to specifications (*2*, *3*)
4 used all-metal valve
2 used Hershberg-Southworth valve (*1*)

CONNECTION OF NITROMETER TO STOPCOCK OR NEEDLE VALVE

1 used glass seal with cement
3 used ℸ (standard taper) joints
All others used rubber tubing having inside diameter of 1 to 3 mm., and outside diameter of 9 to 11 mm.

8. Nitrometer

35 used those according to specifications (*3*)
7 used their own design
43 used stem divisions of 0.01 ml.
45 used manufacturers' calibration values
4 used a water jacket
49 used a special clamp to hold reading lens
15 used a leveling bulb according to specifications (*3*)

9. Thermometer

61 used thermometers
23 measured temperature in funnel
34 measured temperature alongside stem
40 used thermometers calibrated in 0.1° C.
12 used thermometers calibrated in 0.25° C. or more

10. Long Furnace

There were 10 furnaces of individual design and 45 commercial

49 were electrically heated
24 used Nichrome wire
9 used Chromel wire
2 used platinum wire

13 furnaces were less than 18 cm. long
24 were 19 to 20 cm.
14 were 20 to 25 cm.
20 were rectangular
27 were round

20 were heated to 500° to 650° C.
9 were heated to 650° to 800° C.
9 were heated to less than 500° C.

28 furnaces were heated to 500° to 700° C. in region adjacent to sample
27 furnaces had average temperature of 500° to 700° C.

52 furnace temperatures were measured by means of thermocouple
21 measured temperature inside empty furnace
28 measured temperature inside empty combustion tube
12 of furnaces had built-in temperature measuring devices
2 furnaces had automatic temperature control

11. Sample Burner

42 used gas burners
19 used Bunsen burners
34 used gauze around combustion tube
18 used electric burners
10 were from 61 to 88 mm. long
6 were from 80 to 100 mm. long
12 had Nichrome wire elements

6 were heated to 600° to 700° C.
11 were heated to 700° to 800° C.
18 used a thermocouple
36 sample burners were operated manually
15 had motor-driven units
9 were home-made
14 burners were moved at constant rate
16 burners traveled 15 to 100 mm.
14 burners traveled 100 to 150 mm.
18 burners traveled distance in 11 to 20 minutes
11 burners required 21 to 30 minutes
4 burners required 41 to 50 minutes
1 burner required 50 to 60 minutes

13 burned sample once
35 burned sample twice

12. Copper Oxide

34 stored copper oxide in a glass bottle
52 used sieves
27 sieves were from 3 to 6 inches in diameter
27 used 21- to 40-mesh sieves
19 used 81- to 100-mesh sieves

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DDT—Correction

A colorimetric analytical method for DDT [Schechter, Soloway, Hayes, and Haller, IND. ENG. CHEM., ANAL. ED., **17**, 704 (1945); Schechter, Pogorelskin, and Haller, *Ibid.*, **19**, 51 (1947)] failed to caution analysts to dry the acetone-extracted cotton in open air before completion of oven drying to avoid the danger of explosion. The authors wish to add this precaution, which applies to any material that can give off an appreciable amount of combustible vapor.

M. S. SCHECHTER

m-Cresoxyacetic Acid, a Selective Reagent for Zirconium

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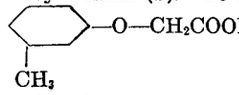
REAGENTS for the determination of zirconium are many, but few are selective. The best known is mandelic acid, recommended by Kumins (*2*). To this may be added *m*-cresoxyacetic acid,  , a reagent that is easily prepared and purified (*1*). From solutions 0.20 to 0.25 *N* in hydrochloric acid, zirconium can be precipitated quantitatively in the presence of a number of elements, including thorium. This paper describes the use of this reagent for the selective precipitation of zirconium. The precipitate is voluminous and settles down quickly. As little as 2 mg. of zirconium oxide gives a precipitate large enough for convenient handling.

EXPERIMENTAL

Estimation of Zirconium. A stock solution of pure zirconyl chloride in 0.1 *N* hydrochloric acid was standardized by precipitation with cupferron (*4*). Aliquots of this solution were estimated with *m*-cresoxyacetic acid. The results are shown in Table I.

Effect of Acid. The reagent precipitates thorium, ferric iron, titanium, and stannous tin as well as zirconium in neutral or slightly acid solution. In a study of the selective precipitation of zirconium in the presence of these elements, the solubility of zirconium *m*-cresoxyacetate in hydrochloric acid was investigated by determining the zirconium oxide obtained from standard solutions in various acid concentrations. It has been observed that the precipitation of zirconium is quantitative in solutions up to 0.25 *N* in hydrochloric acid. Thus a convenient free acid concentration at which separation can be attempted is 0.20 to 0.25 *N*.

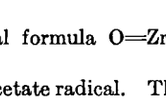
Composition of Precipitate. Ignition tests with the precipitate formed in solution 0.20 *N* in hydrochloric acid pointed to the association of one molecule of *m*-cresoxyacetic acid with each zirconium atom. These results, which showed variations of the order of 3% from sample to sample, conformed to the empirical formula  , where R represents the *m*-cresoxyacetate radical. The precipitate is thus a basic salt of slightly varying composition which precludes its direct weighing.

Table I. Determination of Zirconium in Pure Solutions

Expt. No.	ZrO_2 Taken, Gram	ZrO_2 Found, Gram	Difference, Mg.
1	0.0976	0.0976	...
2	0.0976	0.0975	−0.1
3	0.0732	0.0733	+0.1
4	0.0732	0.0730	−0.2
5	0.0488	0.0488	...
6	0.0488	0.0488	...
7	0.0244	0.0245	+0.1
8	0.0122	0.0124	+0.2
9	0.0041	0.0042	+0.1
10	0.0020	0.0018	−0.2

PROCEDURE

Single Precipitation. To the solution containing not more than 0.1 gram of zirconium oxide, add the calculated quantity of 2 *N* hydrochloric acid to give a free acid concentration of 0.20 *N* in a total volume of 200 ml. and 10 grams of solid ammonium nitrate, then dilute to 100 ml. Boil. Add 2 grams of *m*-cresoxyacetic acid in 100 ml. of boiling water with continuous stirring.

Table II. Separation of Zirconium from Other Elements

(ZrO_2 taken, 0.0488 gram)

Expt. No	Impurity Added, Gram		ZrO_2 Found, Gram	Difference, Mg.
	Concentration of Free Acid = 0.20 *N* HCl			
1	NiO	0.8220	0.0487	−0.1
2	BeO	0.4330	0.0488	..
3	Al_2O_3	0.8420	0.0488	..
4	U_3O_8	0.5110	0.0488	...
5	MnO	0.1894	0.0488	...
6	CaO	0.2548	0.0489	+0.1
7	BaO	0.4100	0.0488	...
8	R_2O_3 [a]	0.4330	0.0488	...
9	Fe_2O_3	0.8664	0.0489	+0.1
10 A [b]	V_2O_4	0.2110	0.0492 [c]	+0.4
10 B [d]	V_2O_4	0.2110	0.0488	...
11 A [b]	Cr_2O_3	0.4346	0.0490 [c]	+0.2
11 B [d]	Cr_2O_3	0.4346	0.0488	...
12 A [b]	SnO	0.2380	0.0520	+3.2
12 B [d]	SnO	0.2380	0.0488	...
13	ThO_2	0.0980	0.0488	...
14	ThO_2	0.1960	0.0489	+0.1
15 A [b]	TiO_2	0.1143	0.0514	+2.6
15 B [d]	TiO_2	0.1143	0.0489	+0.1
	Concentration of free acid = 0.25 *N* HCl			
16	ThO_2	0.1960	0.0487	−0.1
17	ThO_2	0.1960	0.0486	−0.2
18 A [b]	TiO_2	0.1143	0.0510	+2.2
18 B [d]	TiO_2	0.1143	0.0486	−0.2
19 A [b]	SnO	0.2380	0.0515	+2.7
19 B [d]	SnO	0.2380	0.0486	−0.2

[a] Mixture of ceria, lanthana, praseodymia, and neodymia.
[b] Single precipitation.
[c] Residue is slightly colored.
[d] Double precipitation.

Table III. Analysis of Zircon

Sample	Cupferron Method		*m*-Cresoxyacetic Acid Method	
	Sample, g.	ZrO, %	Sample, g.	ZrO_2, %
1	0.1034	65.02	0.1016	65.08
2	0.1145	65.11	0.0984	65.14
3	...	...	0.0972	65.01
	Mean	65.06	Mean	65.08

A voluminous flocculent precipitate results. Continue to boil for 5 minutes and then set aside. Filter through a Whatman No. 42 filter, wash first with hot 0.1% solution of the precipitant in 0.20 *N* hydrochloric acid, then with water, ignite, and weigh as ZrO_2.

Double Precipitation. In the presence of titanium, stannous tin, vanadyl or chromium ion, a double precipitation is necessary. Then do not attempt to wash the precipitate completely. Transfer the precipitate and the filter to the original beaker and dissolve by digesting with 1 to 1 hydrochloric acid on a water bath. Dilute, then adjust the amount of free acid to about 0.20 *N*, and proceed as above.

Separation of Other Impurities. All impurities added in the form of chlorides or nitrates are calculated to the oxides. Table II shows the results obtained.

OBSERVATIONS

Thorium is not precipitated by the reagent until the free acid concentration falls below 0.10 *N*.

When present singly, titanium and stannous tin are not precipitated in acid solutions 0.10 *N* and stronger, but in the presence of zirconium small quantities are carried down even in 0.25 *N* free acid. The separation is, however, complete in a double precipitation.

Vanadyl and chromium are not precipitated from neutral solutions, but contaminate the zirconium precipitate even at 0.20 *N* acid concentration. The amount carried down is so small that the weight of zirconia is not significantly higher,

but the ignited residue is slightly colored. On a second precipitation the color disappears.

Ferric iron is only partially precipitated in neutral solutions.

Beryllium, aluminum, nickel, calcium, barium, uranyl, and trivalent rare earths (cerite group) are not precipitated.

Sulfate interferes with the determination of zirconium.

ANALYSIS OF ZIRCON

The method was checked by determining zirconia in a zircon ore from Travancore, India, which was found on qualitative analysis to contain silica, titania, and ferric oxide as well as zirconium. Thorium, aluminum, and the rare earths were not present in detectable amounts. The ore was fused with borax (*3*) and after removal of silica, zirconium was determined by double precipitation with *m*-cresoxyacetic acid in 0.20 *N* hydrochloric acid. The results are shown in Table III. For comparison, values obtained with the cupferron method (*4*) are included.

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Fluorometric Determination of Benzil

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IN AN investigation of miticides for use in clothing, a method was required for the determination of small amounts of benzil impregnated in cloth. Attempts to find a suitable colorimetric method for this new effective miticide (*1, 2, 7*) were unsuccessful (*4*). An adaptation of a gravimetric method (*5*) was found to be accurate for larger quantities of benzil in pure finish cloth (*4*), but impurities which are soluble in ethyl alcohol and insoluble in dilute hydrochloric acid interfered and the method was not suitable for trace amounts. Application of ultraviolet absorption measurements at the strong absorption band of benzil (260 mμ) was limited because of marked interferences in cloth containing ultraviolet-absorbing material such as certain sizing preservatives.

The condensation reaction (*3*) of diketones such as benzil and nitrobenzils with alkylated aminophenols to form blue-red dyestuffs with yellow-red fluorescence was utilized in the method described here. In the procedure developed, benzil is determined photofluorometrically as a condensation product with *m*-diethylaminophenol. It was also observed that very small amounts of alkylated aminophenols can be detected by condensation with an excess of benzil.

APPARATUS

Klett photofluorometer, Model 2070, with matched tubes and filters 597 and 351. Beckman quartz spectrophotometer, Model CUV, range 220 to 1000 mμ, with interchangeable hydrogen discharge lamp in housing, power supply unit, and a pair of fused silica absorption cells with borosilicate glass covers.

REAGENTS

Benzil, technical grade material, purified by recrystallization from 95% ethyl alcohol. Analysis: C, 79.9%; H, 4.77% (calculated for benzil, $C_{14}H_{10}O_2$: C, 79.98%; H, 4.79%).

Reagents for fluorometric method. *m*-Diethylaminophenol, recrystallized from 95% ethyl alcohol. Reference solution, 0.0004 gram of fluorescein in 1 liter of water.

Reagents for gravimetric determination. 2,4-Dinitrophenylhydrazine solution made by saturating 2 *N* hydrochloric acid with 2,4-dinitrophenylhydrazine, 2 *N* hydrochloric acid, and 95% ethyl alcohol.

DEVELOPMENT OF FLUOROMETRIC METHOD

Investigations were conducted to determine whether the condensation reaction (*3*) could be adapted as a quantitative method for the estimation of benzil. Exploratory work with several aminophenols indicated that a compound melting below 100° C. would give the best results. *m*-Diethylaminophenol (melting point 78° C.) was found to be a satisfactory reagent when condensed with benzil at 100° C. for 90 minutes. Two to 4 moles of the aminophenol are required for each mole of benzil (*3*). Recoveries of 98% and better were obtained on purified samples of benzil by this method.

Unsized, undyed cotton pure-finish herringbone twill cloth was impregnated with known quantities of benzil. The miticide was then extracted with ethyl alcohol and determined by the fluorometric method. Recoveries of 97% or better were obtained. With benzil which had not been impregnated in cloth recoveries of 98% or better were obtained.

Table I. Comparison of Fluorometric with Gravimetric and Ultraviolet Absorption Methods for Determination of Benzil in Cloth

Sample No.	Fixative	Benzil, % Fluorometric	Gravimetric	Ultraviolet absorption measurement
1	Chlorinated paraffin	5.17	5.10	5.35
2	Chlorinated paraffin	5.20	5.02	5.29
3	Chlorinated paraffin	2.15	2.07	2.08
4	Chlorinated paraffin	0.07	..	0.07
5	Chlorinated paraffin	0.01	..	0.01
6	None	7.66	7.47	7.61
7	None	0.17	0.19	0.18
8	None	0.25	0.27	0.25
9	None	0.76	0.80	0.78

The results given in Table I were obtained by application of the following fluorometric method to samples of pilot plant impregnated cloth. As this cloth did not contain material that would interfere in the gravimetric or ultraviolet absorption methods, these methods were used for comparison purposes and are also described below.

Cut the cloth sample into 1-cm. squares and mix to make more homogeneous. Place a 3.000- to 5.000-gram sample of cloth (enough to contain at least 1 mg. of benzil) in the thimble of a Soxhlet extraction apparatus. Extract with 95% ethyl alcohol for 2 hours. If the extract is turbid, let it stand overnight and filter. Make up the extract to a known volume in a volumetric flask with 95% ethyl alcohol, so that a concentration of 0.01 to 0.04 mg. of benzil per ml. is obtained. Using a pipet or microburet, accurately measure 1 ml. of the solution into a test tube. To the same test tube add from a microburet 1 ml. of a 95% ethyl alcohol solution containing 50 mg. per ml. of *m*-diethylaminophenol. Place the test tube over a hot water bath maintained at 100° C. and heat for 90 minutes. (A 3-liter beaker covered by a Transite board with holes large enough to support a test tube by the flare serves as an excellent bath when heated on a hot plate. The water level should be just below the bottom of the tube.) Wash the melt from the test tube into a 100-ml. volumetric flask and make up to volume with 95% ethyl alcohol.

Make measurements on a Klett photofluorometer using filters 597 and 351 with a reference solution of 0.0004 gram per liter of fluorescein. Prepare a curve relating fluorometer reading with

Table II. Fluorometric Determination of Benzil in Cloth Containing Various Impregnating Agents

Additive Impregnated in 2.5 G. Cloth Sample		Benzil Added to Sample, Mg.	Benzil Found, Mg.
Agent	Weight, g.		
Sizing	Unknown	0.025	0.024
Sizing	Unknown	0.025	0.024
Nacconal	0.05	0.025	0.023
Nacconal	0.05	0.025	0.024
Span 80	0.05	0.025	0.025
Span 80	0.05	0.025	0.024
Chlorinated paraffin	0.125	0.025	0.025
Chlorinated paraffin	0.125	0.025	0.024
Polyvinyl alcohol	0.013	0.025	0.021
Polyvinyl alcohol	0.013	0.025	0.016

benzil concentration, using known solutions treated as described above, and make the quantitative determination of the unknown by applying the reading obtained. For best results the calibration curve and unknown samples should be run on the same day, because the aminophenols tend to darken on standing. Run a suitable blank under the same conditions with unimpregnated cloth.

Tests were run using various fixatives, laundering agents, and emulsifiers which might be used in cloth impregnation studies. Of these materials, chlorinated paraffin gave the least interference and polyvinyl alcohol gave the greatest. In most cases, compensation for interferences could be made by use of suitable blanks. Results, corrected by use of blanks, of the fluorometric determination of benzil in the presence of various agents are listed in Table II.

Gravimetric Method. Recoveries of 97 to 98% were obtained in the estimation of benzil by a method for the determination of water-insoluble carbonyl compounds by means of 2,4-dinitrophenylhydrazine (*5*). The method used was essentially the same as described by Iddles *et al.* with the addition of a 2-hour extraction of the cloth sample with 95% ethyl alcohol, followed by the precipitation of the benzil compound from an aliquot portion of the extract.

Ultraviolet Absorption Method. Ethyl alcohol was used as the solvent and absorption measurements were made with a Beckman quartz spectrophotometer. The maximum absorption for benzil in 95% ethyl alcohol occurs at a wave length of about 260 mμ (*6*). Absorption readings were made at 260 mμ and a slit width of 0.5 mm. The Beer-Lambert law holds only approximately for benzil in 95% ethyl alcohol at this wave length. For this reason it was necessary to plot a curve of log I_0/I versus concentration. The cloth samples were extracted with 95% ethyl alcohol and readings were made on concentrations in the range of 0.003 to 0.015 gram per liter.

DISCUSSION

Marked changes in the fluorescence of solutions containing the fluorescent compound took place with large changes of pH. Solutions made alkaline with ammonium or potassium hydroxide showed a great decrease in fluorescence. However, under the conditions of the procedure outlined above, only a slight effect due to small amounts of acidity in the alcohol was noted. Neutralization of the alcohol eliminated this difficulty.

In all applications of the fluorometric method described here, a straight-line curve of Klett photofluorometer readings against milligrams of benzil in concentrations up to 0.03 mg. per ml. was obtained.

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Colorimetric Method for Determination of Traces of Carbonyl Compounds

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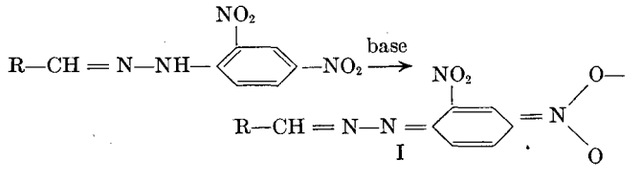
THE addition of a solution of sodium or potassium hydroxide to an alcoholic solution of a 2,4-dinitrophenylhydrazone produces a very intense wine-red color, presumably due to the formation of the resonating quinoidal ion I. A similar quinoidal ion



has been suggested for the colored solution formed when base is added to the phenylhydrazone of a nitroaromatic aldehyde (*1*). This color reaction has been made the basis of a very sensitive method for the estimation of ketosteroids in biological extracts (*2*). Herein is reported the extension of the method to the quantitative determination of traces of aldehydes or ketones in water, organic solvents, or organic reaction products. The method is most useful in the range of carbonyl concentration from 10^{-4} to 10^{-6} molar, wherein few if any other methods give reliable results or are of general application.

Absorption spectra were run on alkaline alcoholic solutions of a number of 2,4-dinitrophenylhydrazones. It was found that the position of the maximum as well as the value of $E_{max.}$ was nearly independent of the structure of the carbonyl compound (with exceptions noted below) and were independent of the concentration of base as long as a sufficient excess was present. The colors formed were relatively stable, although slow fading over a period of several days was noted. Beer's law was obeyed in the concentration range studied. The value of $E_{max.}$ determined for a large number of compounds averaged 2.72×10^4 at 480 mμ. Table I gives more exact values for a number of compounds.

For actual analysis it was found unnecessary to isolate the phenylhydrazone. If it was prepared in solution, using an excess of 2,4-dinitrophenylhydrazine, the addition of base converted the excess reagent to a very light yellow substance, the absorption of which was corrected for by using a blank determination.

PREPARATION OF REAGENTS

Carbonyl-Free Methanol. To 500 ml. of C.P. methanol were added about 5 grams of 2,4-dinitrophenylhydrazine and a few

Table I. Position and Values of $E_{max.}$ for Various Compounds

Compound	Maximum, mμ	$E_{max.} \times 10^{-4}$
Acetaldehyde	478	2.72
Acetone	476	2.66
Acetophenone	480	2.71
Anisaldehyde	480	2.70
Acetylacetone	480	5.42
Acetthienone	480	2.71
Benzaldehyde	481	2.72
Butyraldehyde	480	2.73
Cinnamaldehyde	480	2.70
Cyclohexanone	480	2.69
Cyclopentanone	480	2.68
3,5-Dichlorobenzaldehyde	480	2.70
Furfural	479	2.72
9-Heptadecanone	480	2.68
p-Hydroxyacetophenone	480	2.70
Methyl cyclopropyl ketone	476	2.69
Methyl ethyl ketone	480	2.75
Methyl phenyl diketone	480	5.46

drops of concentrated hydrochloric acid. After refluxing 2 hours, the methanol was distilled through a short Vigreux column. If kept tightly stoppered, the methanol remains suitable for use for several months.

2,4-Dinitrophenylhydrazine Solution. A saturated solution in carbonyl-free methanol was prepared, using 2,4-dinitrophenylhydrazine which had been twice recrystallized from this solvent. This solution should not be used more than a week or two after preparation.

Potassium Hydroxide Solution. Ten grams of potassium hydroxide were dissolved in 20 ml. of distilled water and the solution was made up to 100 ml. with carbonyl-free methanol. This solution will keep indefinitely.

PROCEDURE

The unknown or its solution should not be more than 10^{-3} molar in carbonyl. In such dilute solutions the phenylhydrazone will not precipitate at room temperature. The solution must be neutral or very weakly acidic to prevent precipitation of potassium salts when the base solution is added.

To 1.0 ml. of the unknown or its solution in carbonyl-free methanol were added 1.0 ml. of the 2,4-dinitrophenylhydrazine reagent and 1 drop of concentrated hydrochloric acid. The tube was loosely stoppered and heated in a water bath at 50° for 30 minutes or at 100° C. for 5 minutes. After cooling, 5.0 ml. of the potassium hydroxide solution were added. The almost black solution which resulted rapidly cleared to the characteristic wine-red color. A blank determination was made simultaneously using 1.0 ml. of the carbonyl-free methanol in place of the sample.

The optical density of the solution was determined using a Beckman Model DU spectrophotometer. The instrument was adjusted for 100% transmittance for the solution from the blank determination, no further correction for the blank being necessary. The measurement was made at 480 mμ and the calculations were made using the average value of $E_{max.}$ In later work the instrument was standardized using acetophenone and a graph was constructed to allow direct reading of carbonyl concentration from the observed optical density.

DISCUSSION

The method has been found to be applicable to a large number of aldehydes and ketones, both aliphatic and aromatic, as well as to some diketones. The only interfering structures so far encountered are nitroaromatic groups and conjugation of the chalcone-type ketones. Compounds containing such groups can still be determined by using the same compound for standardization. Accuracy of the order of 2 parts per hundred was obtained in the range of 5×10^{-6} to 10^{-4} molar carbonyl. Carbonyl concentrations as low as 5×10^{-7} molar can be detected qualitatively.

The authors have successfully used the method to determine carbonyl compounds in water solutions, and organic solvents such as alcohols, acetic acid, ethers, and ethyl acetate; to the detection and estimation of small quantities of carbonyl compounds formed in certain rearrangement reactions (*3*, *4*); to the qualitative identification of aldehydes and ketones in an organic qualitative analysis course (for this purpose the intense color due to larger concentrations of carbonyl compounds makes it easy to distinguish visually between trace impurities and a major component); and to determine the number of carbonyl groups in a compound of known molecular weight (*4*).

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RECEIVED May 4, 1950.

Purification of Methyl Acetate and Ethyl Acetate

CHARLES D. HURD AND JAMES S. STRONG
Northwestern University, Evanston, Ill.

PURE ethyl acetate is required in some analytical operations, such as the separation of soluble sodium perchlorate from insoluble potassium perchlorate (*5*). Water and ethyl alcohol are the usual impurities to be anticipated. However, for most methods in which ethyl acetate is employed in quantitative analysis, small quantities of water and alcohol are not prohibitive.

The method of purification which seems to have been generally adopted (*1–5*) for both methyl and ethyl acetates is the use of phosphorus pentoxide. Apparently the use of acetic anhydride for this purpose has been overlooked. The latter reagent is suitable not only for the ordinary ester of 97 to 98% purity but also for esters containing much higher amounts of alcohol. An obvious advantage is that the alcohol content becomes converted into the desired ester. This method was tested and found to be suitable. Most of the acetic acid which was formed was removable by distillation. Then, treatment with anhydrous potassium carbonate and redistillation gave essentially pure ester.

One liter of ordinary methyl acetate (d_{25}^{25} 0.9309) was refluxed for 6 hours with 85 ml. of acetic anhydride, then was distilled (boiling point 56.0–56.5°) through a Vigreux column. The distillate was shaken with 20 grams of anhydrous potassium carbonate for about 10 minutes and redistilled. The density of this material (d_{25}^{25}) was 0.9284, comparing with Perkin's (*3*) value of 0.92825. Careful determination of the saponification equivalent of this material showed it to have a purity of 99.87 ± 0.02%. About the same results were obtained if a few drops of sulfuric acid were added to the acetylation mixture.

A mixture of 1 liter of ethyl acetate (98.01% purity, as determined by saponification equivalent), 55 ml. of acetic anhydride, and 6 drops of concentrated sulfuric acid was refluxed for 4 hours, then processed as above. After treatment with potassium carbonate and redistillation, the purity of the ethyl acetate was 99.65% as shown by saponification equivalent.

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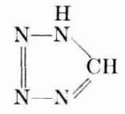
RECEIVED May 31, 1950.

CRYSTALLOGRAPHIC DATA

Contributed by WALTER C. McCRONE, DONALD GRABAR, AND EUGENE LIEBER
Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

42. Tetrazole



Structural Formula for Tetrazole

TETRAZOLE can be recrystallized from ethyl acetate and other common organic solvents, but the best crystals for both optical and x-ray studies are obtained by slow sublimation (at about 50° C.) onto a microscope slide. Characteristic crystals by sublimation are shown in Figure 1; crystals grown from the melt are shown in Figure 2. An orthographic projection of a typical crystal of tetrazole is shown in Figure 3. All crystal interfacial angles were measured microscopically and are subject to ±3° error. This accounts also for the rather bad agreement between the observed and calculated density.

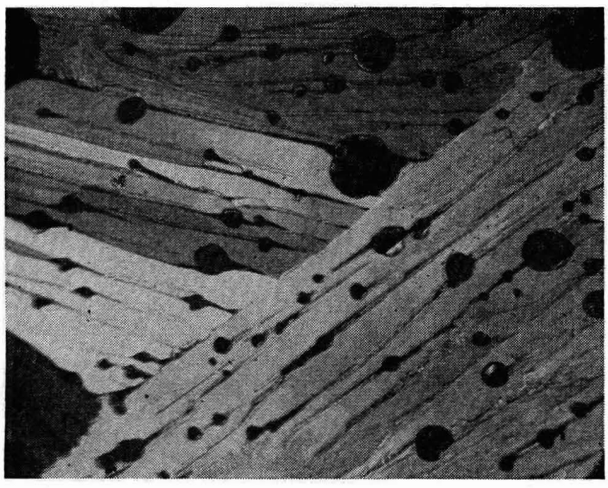


Figure 1. Crystals of Tetrazole Sublimed onto Microscope Slide

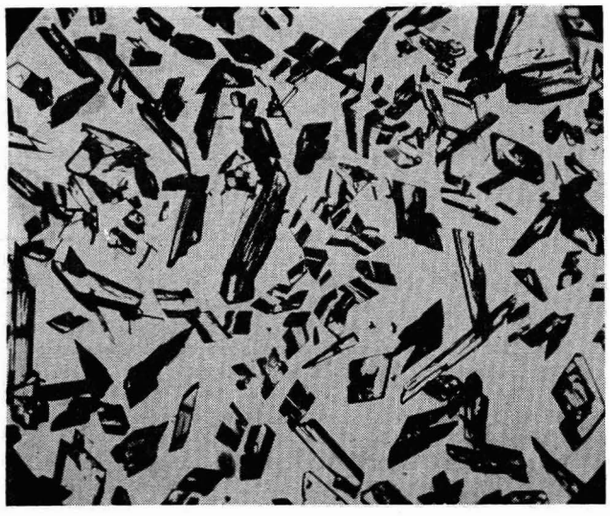


Figure 2. Crystals of Tetrazole from Melt
Crossed Nicols

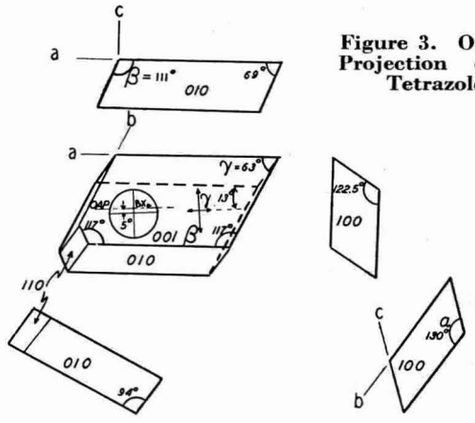


Figure 3. Orthographic Projection of Typical Tetrazole Crystal

CRYSTAL MORPHOLOGY

Crystal System. Triclinic.

Form and Habit. Plates flattened parallel to and usually lying on 001, showing the pinacoids, {001}, {010}, {100}, and occasionally the prisms, {110}.

Axial Ratio. $a:b:c = 0.915:1:0.687$.

Interfacial Angles (Polar). 001 Λ 100 = 86°; 001 Λ 010 = 57.5°; 100 Λ 010 = 106°.

Crystal Angles. $\alpha = 130°$; $\beta = 111°$; $\gamma = 63°$.

X-RAY DIFFRACTION DATA

Cell Dimensions. $a = 5.00$; $b = 5.46$; $c = 3.75$.

Formula Weights per Cell. 1.

Formula Weight. 70.06.

Density. 1.406 (flotation); 1.632 (x-ray).

Principal Lines

d	I/I_1	d	I/I_1
4.35	0.77	1.727	0.04
3.91	0.32	1.703	0.10
3.38	0.60	1.676	0.02
3.20	1.00	1.649	0.03
2.68	0.10	1.603	0.09
2.62	0.06	1.572	0.03
2.48	0.10	1.553	0.04
2.33	0.06	1.536	Very weak
2.27	0.05	1.492	0.03
2.17	0.15	1.463	0.02
2.04	0.06	1.438	0.02
1.943	0.08	1.403	Very faint
1.870	0.04	1.364	Very faint
1.799	0.08	1.312	Very faint
1.773	0.06	1.195	Very faint
1.748	0.04	1.177	Very faint

OPTICAL PROPERTIES

Refractive Indexes (5893 A.; 25° C.). $\alpha = 1.388 \pm 0.005$. $\beta = 1.595 \pm 0.002$. $\gamma = 1.660 \pm 0.002$.

Optic Axial Angles (5893 A.; 25° C.). $2V = 51°$. $2E = 90°$.

Dispersion. $v > r$ very slight.

Sign of Double Refraction. Negative.

Acute Bisectrix. Almost ±001.

Extinction. $\gamma \Delta a = 13°$ on 001.

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.54$. R(calcd.) = 19.7 (imidine value for N's). R(obsd.) = 15.6.

FUSION DATA. Tetrazole melts without decomposition at 155.5° (equilibrium micro melting point) and crystallizes without supercooling to form flat rods with oblique extinction (ca. 15°) and showing centered BX_a figures. Negative, $v > r$, $2E = 90°$.

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Section, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

Book Reviews

Analytical Chemistry of the Manhattan Project. *Clement J. Rodden*, Editor-in-Chief. xx + 748 pages. McGraw-Hill Book Co., Inc., 330 West 42nd St., New York 18, N. Y., 1950. Price, $6.75.

Analytical chemists have looked forward to the appearance of this volume, one of the National Nuclear Energy Series. It is an integrated compilation of methods developed in all the analytical research laboratories of the Manhattan Project. The book is divided into two parts, the first dealing with the analytical chemistry of the individual elements studied on the project and the second with special techniques such as electrolytic, photometric, electrometric, spectrochemical, and radiochemical. In Part I, approximately 200 pages are devoted to uranium and thorium. The analytical chemistry of these two elements is covered very thoroughly. Methods of separation and determination of both macro and micro quantities are given in detail; selected procedures for ore and mineral analysis are presented. The other elements are treated more briefly. To a large extent the determination of traces of these elements was required in the examination of project materials and such methods make up a considerable portion of this section. Radiochemical methods are covered only superficially, as they are treated in detail elsewhere in the series.

Although it is stated in the introduction that "the methods that were developed were in the majority of cases based on those previously published and in many cases were but adaptations for specific purposes," the analyst will discover much that is new and interesting. There is hardly anyone concerned with the analysis of inorganic materials who will not find something of value to him in this book.

E. B. Sandell

Symposium on Rapid Methods for the Identification of Metals. 84 pages. American Society for Testing Materials, Philadelphia, Pa. Price, $1.75.

This publication covers the most recent developments in the field of rapid methods for the identification of metals. The techniques included, some of which have not been described previously, should prove of great value especially where the testing must be done in the field—that is, where the laboratory must be taken to the sample. Those concerned with scrap problems, stocking of various metal products, sorting of various alloy parts, should find this publication of interest and help. The symposium was sponsored by Committee E-3 on Chemical Analysis of Metals, American Society for Testing Materials.

The nine comprehensive papers in the publication and their authors are: "Development, Present State, and Outlook of Spot Test Analysis," F. Feigl, Laboratorio da Produção Mineral, Ministerio da Agricultura, Rio de Janeiro, Brazil; "Electro Spot Testing and Electrography," H. W. Hermance and H. V. Wadlow, Bell Telephone Laboratories; "Instruments for Rapid Metal Identification," R. R. Webster, Jones & Laughlin Steel Corp.; "Separating Alloys by Relative Spot Tests," H. Kirtchik, General Electric Co.; "Rapid Methods for the Identification of Copper-Base Alloys," R. P. Nevers, American Brass Co.; "Rapid Identification of Metal Finishes," A. Lewis and D. R. Evans, Western Electric Co.; "Examination of Plated and Protective Coatings by Electrographic Analysis," N. Galitzine and S. E. Q. Ashley, General Electric Co.; "Field Test Kit and Procedure for Use in Rapid Identification of Some Nickel Alloys and Stainless Steels," H. B. Lea, Eastman Kodak Co.; "Rapid Tests for Identifying Alloy Steels," E. C. Kirkham, University of Utah.

The Electron Microscope. *V. E. Cosslett.* viii + 128 pages. Interscience Publishers, Inc., 250 Fifth Ave., New York 1, N. Y. Price, $1.25.

Recently the literature concerning the electron microscope has been expanding rapidly in both theoretical and practical elementary fields. "The Electron Microscope," by Cosslett, is certainly one of the better treatments from an elementary viewpoint of this rather technical subject. The English author, a recognized authority on this subject, has addressed the volume to a person of reasonable intelligence, but with little knowledge of optics, either light or electron. The book is clearly written in a very readable style, and what is most remarkable, without the use of a single mathematical equation, a difficult thing to do on such a topic.

The author takes a very realistic view of the subject, leaning toward conservatism on controversial points particularly in the chapter on future possibilities of the electron microscope. Although the book is pocket size, the subject is very completely covered with little unnecessary detail. Inexpensive paper has been used for the book, except for the excellent reproduction of a small collection of representative electron photomicrographs on a good grade of coated stock. An adequate number of line diagrams are included, but the bibliography is almost nonexistent. The few references that are included, however, lead to a very complete general treatment of the subject of the electron microscope.

This is a well-written elementary book by an authority in the field of electron optics and can be read to advantage even by an experienced electron microscopist.

Ernest F. Fullam

The Analyst's Calendar

Symposium on Molecular Structure and Spectroscopy

A Symposium on Molecular Structure and Spectroscopy will be held at the Department of Physics, Ohio State University, Columbus, Ohio, June 11 to 15, 1951. There will be discussions of the interpretation of molecular spectroscopic data as well as methods of obtaining such data, and sessions devoted to phases of spectroscopy of current interest. The symposium will be sponsored jointly by the Graduate School and the Department of Physics and Astronomy at Ohio State University and the Division of Chemical Physics of the American Physical Society.

A dormitory will be available for those who wish to reside on the campus during the meeting.

Further information is available from Harald H. Nielsen, Department of Physics, Ohio State University, Columbus 10, Ohio.

Instrument Society of America. Pittsburgh Section and Carnegie Institute of Technology. Pittsburgh, Pa., March 28 and 29. New Jersey Section. Newark, N. J., April 3

U. S. National Committee of the International Commission for Uniform Methods of Sugar Analysis. Boston, Mass., April 5 and 6

Scientific Apparatus Makers Association. Greenbrier Hotel, White Sulphur Springs, W. Va., April 15 to 18

Symposium on Molecular Structure and Spectroscopy. Ohio State University, Columbus, Ohio, June 11 to 15

Fourth Annual Summer Symposium. Washington, D. C., June 14 to 15

AIDS FOR THE ANALYST . . . . .

Modified Syringe for Extraction with Solvents of Low Density. A. I. Medalia and R. W. Stoenner, Brookhaven National Laboratory, Upton, L. I., N. Y.

MANY extraction procedures of value in analytical chemistry use a solvent, such as ethyl or isopropyl ether, which is less dense than the aqueous medium from which the substance in question is being extracted. Because quantitative separation can generally be obtained only by repeated extraction, it is necessary to transfer the aqueous phase back and forth from one separatory funnel to another, each time with rinsing; or if only one separatory funnel is used, to reserve the aqueous phase in a separate container and transfer it back to the funnel after removal of the organic phase. During these operations the organic phase generally becomes contaminated with aqueous emulsion which clings to the separatory funnel after drainage of the aqueous phase. To avoid this cumbersome procedure, various assemblages of separatory funnels have been proposed (*2*), which, however, are both cumbersome and fragile.

In Figure 1 is shown a simple apparatus by means of which repeated extractions may be carried out without transfer of the denser phase out of the apparatus. The apparatus consists simply of a syringe with an eccentric tip, to which are sealed a stopcock and an outlet tube. The spherical joint shown in the figure may be omitted for the sake of economy; however, its use gives greater flexibility, and permits extraction with solvents denser than water. Syringes with eccentric tip are commercially available in various sizes, and are generally constructed of borosilicate glass. Because there is some variation in manufacture, only the more closely fitting syringes should be selected.

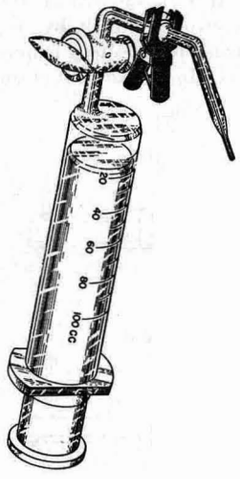


Figure 1

The aqueous solution is introduced by placing the solution in a container with a pointed bottom, and then sucking it into the syringe; rinsings of the container are then introduced in the same manner. The aqueous solution may also be pipetted directly into the open syringe barrel, clamped in a nearly horizontal position, with care to avoid introduction of this solution into the capillary tip during insertion of the plunger. (If the open syringe barrel were held vertically, a portion of the solution would enter the capillary tip and be lost when the plunger was inserted, as this can be done only with the stopcock open.) The organic solvent is next sucked in through the outlet tube by drawing out the plunger. Some air is also drawn in, the stopcock is closed, and then the syringe is shaken. The plunger will not drop out during shaking; if pressure is generated by volatilization of the organic solvent, the plunger is forced out a short distance until, according to the gas law, the volume of the gas space is increased by a factor of $1 + P$, where P is the vapor pressure of the solvent in atmospheres. The syringe is then held as shown in Figure 1, and after separation of the phases, the lighter phase is forced out through the outlet tube. The eccentric tip is kept at the top, so that none of the organic phase is trapped. The amount of liquid delivered can be closely controlled; and the organic solvent is expelled free from water. More organic solvent is then sucked in through the delivery tip, thus rinsing the contents of the tip back into the syringe, and further extractions are performed as before. Finally, the aqueous phase may be forced out through the outlet tube and the syringe rinsed by sucking in water and air, shaking, and again forcing out the water.

It may be desirable to use the syringe for extractions with solvents denser than water—for example, different elements may be extracted successively from the same solution, such as ferric chloride with ether, then copper carbamate with carbon tetrachloride. For extraction with dense solvents, the same procedure is used as with light solvents, but the outlet tube is turned in the opposite direction from that shown, and the whole syringe is inverted to permit the dense solvent to be forced out through the outlet tube. The portion of the solution (in the dense solvent) left in the outlet tube below the stopcock is not lost, as with conventional separatory funnels, but is drawn back into the syringe when fresh solvent is sucked in.

Experimental. As a test of the completeness of extraction with this apparatus, ferric chloride was extracted with isopropyl ether, as recommended by Dodson, Forney, and Swift (*1*).

A solution of 0.0964 *M* ferric chloride in 7.87 *N* hydrochloric acid was analyzed by reduction with stannous chloride and mercuric chloride, followed by titration with ceric sulfate. Aliquots of 25 ml. were placed in the syringe and extracted with three successive portions (20 to 25 ml.) of reagent grade isopropyl ether. Dilute hydrochloric acid was added to the combined extracts, the ether was boiled off, and the remaining solution was analyzed as before. Taken, 134.8 mg. of iron; found, 134.9 mg. The aqueous phase, combined with three rinsings of the syringe with hydrochloric acid, was tested for ferric iron with thiocyanate. It was found that less than 0.01 mg. of iron was present.

To check the sharpness of separation of the two phases, a solution of 1.5 grams of cupric chloride dihydrate in 7.87 *N* hydrochloric acid was extracted once with isopropyl ether. Water was added to the extract, the ether was boiled off, and the copper was determined with diethyldithiocarbamate. Found, 0.02 mg. of copper.

LITERATURE CITED

(1) Dodson, R. W., Forney, G. J., and Swift, E. H., *J. Am. Chem. Soc.*, 58, 2573 (1936).
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RESEARCH carried out under the auspices of the Atomic Energy Commission.

Hydrocarbon Absorption by Stopcock Lubricants. M. H. Polley, Godfrey L. Cabot, Inc., Boston, Mass.

IN THE measurement of calorimetric heats of adsorption of hydrocarbons on carbon black at 100° C. (*5*), mercury cutoffs in the adsorption apparatus, which were used successfully at room temperature (*1, 6*), were rejected because of the appreciable vapor pressure of mercury at the higher temperature. The use of glass stopcocks in such a system requires a lubricant of low vapor pressure, insoluble in hydrocarbon vapor and of good consistency at both room temperature and 100° C. After considerable investigation of lubricants recommended for use with hydrocarbon vapors (*2, 4*), a cellulose acetate grease made by a slight variation of the method of Pearlson (*3*) was found to possess excellent working characteristics in the presence of hydrocarbons at elevated temperatures.

Preparation of Cellulose Acetate Lubricant. To 45 grams of 200 molecular weight polyethylene glycol 6.0 grams of commercial cellulose acetate were added and the mixture was heated on an oil bath at 140° C. about 25 minutes. Then 0.6 gram of cellulose acetate was added and heating was continued until the preparation was homogeneous. About 29 grams of citric acid, heated on

an oil bath to 190° C., were added and the mixture was heated at 190° C. for another hour, with frequent stirring. The grease was cooled and dehydrated in a vacuum desiccator. At room temperature it became extremely viscous and tacky.

EXPERIMENTAL PROCEDURE AND RESULTS

In conjunction with the search for a suitable stopcock lubricant, a separate testing procedure was adopted to determine the extent of hydrocarbon absorption by the lubricant. The apparatus employed for this study was similar to that developed for the measurement of heats of adsorption at 0° C. (*1*), using mercury cutoffs.

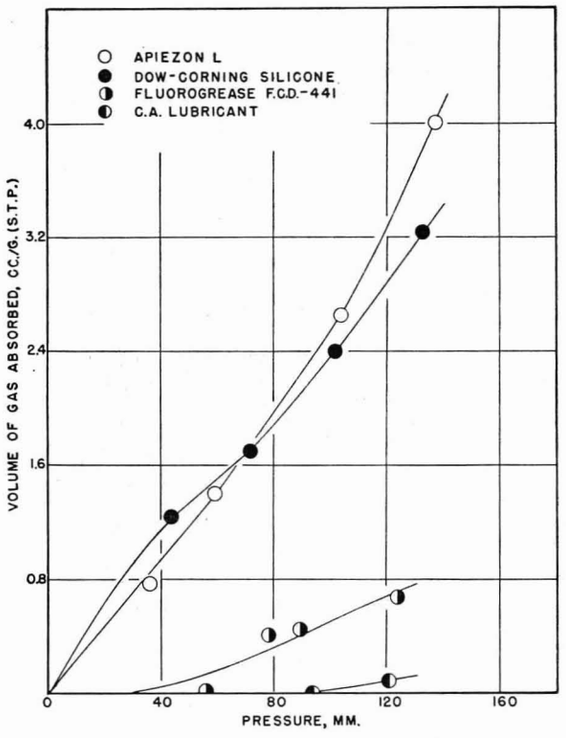

Figure 1. *n*-Hexane Absorbed on Stopcock Lubricants at 100° C.

Approximately 0.10 gram of grease was placed in an adsorption cell and outgassed at 100° C. to free the sample of occluded air and moisture. Because of widely different temperature-viscosity relationships, the extent of surface and thickness of the sample varied slightly. The dead space was determined with helium and the sample evacuated once more. The *n*-hexane, obtained from Humphrey-Wilkinson, Inc., New Haven, Conn., and stated to be of at least 95 mole % purity, was further purified by several bulb-to-bulb distillations. During the experiment the adsorption cell was immersed in a boiling water bath. Four high-vacuum greases were investigated in this manner, including Apiezon L, Dow-Corning silicone, a perfluoro grease (*2*) supplied by the Organic Chemicals Division of the Du Pont Co., and the cellulose acetate lubricant.

The results of the absorption study are shown in Figure 1, in which the isotherms at 100° C. are plotted for *n*-hexane vapor on the four stopcock lubricants. As the sorption process is very slow, these values only approximate the true equilibrium readings. Each point was obtained after at least 20 minutes' exposure. After this period, the rate of pressure drop had decreased to less than 0.1% per minute. It may be seen from these isotherms that Apiezon L and Dow-Corning silicone lubricants slowly absorb large quantities of hydrocarbon vapor. The perfluorocarbon fraction was fairly satisfactory in this respect, but in use the stopcock film became striated within a short time at 100° C. The cellulose acetate preparation, on the other hand, has a low vapor pressure, good consistency at high temperature, and negligible hydrocarbon absorption. It was found, however, that at the end of a 14-hour calorimetric run there was a slight breakdown of the grease, necessitating the cleaning and regreasing of the stopcocks.

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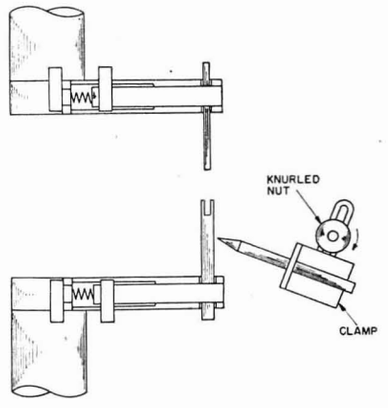
Drying Spectrographic Electrodes Directly on Arc Stand. A. J. Mitteldorf, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

OCCASIONALLY a conventional 1.5-meter A.R.L. spectrograph is used to determine the silicon content of specially distilled water. As the concentration of silicon involved is very small (less than 0.0005%), contamination, especially from dust in the air, presents a large problem.

In order to reduce this contamination a scheme for drying electrodes directly by an arc on the arc stand has been devised, which reduces to a minimum handling of the graphite electrodes and also the amount of time that the electrodes are exposed.



After each electrode is preburned, a measured quantity (0.1 ml.) of water sample is placed in its cup, using a micropipet, while the electrode remains in its clamp. A self-ignited direct current arc is then directed to the side of the electrode about 2 cm. below the top of the cup. Applied at 12 amperes for about 1 second, the heat from the arc is conducted to the water in the cup and causes it to evaporate rapidly. If this arc is applied for too long a time, the water may sputter out of the cup.

The universal arc-spark stand (A.R.L. Model 2061) is well suited for drying electrodes by this technique. The electrical connection normally used for the Petrey stand serves to support a swiveling clamp, which in turn holds a length of pointed, high purity graphite rod 0.25 inch in diameter. To dry the water out of the cupped electrode, the upper electrode is raised about 2 cm. (to prevent arcing to it) and the side electrode is pivoted into the position shown in the diagram. Because the gap between the side and main electrodes (about 2 mm.) is shorter than that between the upper and main electrodes, the arc is struck between the former two by the Multisource. After the arc from the side electrode has dried the main electrode, the side electrode is swung away and the upper electrode is brought back into position for arcing the material and exposing the spectrogram.

Using this scheme for drying electrodes, calcium and magnesium as well as silicon contamination has been reduced to such an extent that the limiting source of contamination is now the electrodes themselves. Even in commercial graphite electrodes of the highest purity, these elements frequently occur as troublesome impurities.

INSTRUMENTATION

A new instrument enables the recording of continuous and accurate curves of any two interdependent functions that can be converted into d.c. signals

by R. H. Müller and S. T. Zenchelsky

THE analytical chemist is becoming increasingly addicted to the automatic recording of data. Well-established examples are the polarograph, recording spectrophotometers for the visible and ultraviolet, x-ray diffraction spectrometers, single- and double-beam infrared spectrophotometers, and the mass spectrometer. Countless recording pH meters and pyrometers are called upon to supply information of indirect analytical interest. More indirect examples are consistomers and plastometers which record physical properties as a function of one or more variables, one of which may be the chemical composition. Much new information is being acquired about classical gravimetric procedures by means of differential thermal analysis, in which the thermal history of a sample is automatically recorded.

Every branch of analytical chemistry could be enriched by the automatic recording of data. Another way of stating this is to say that if the speed of response of the recorder is commensurate with the establishment of equilibrium in the system, one can obtain an "infinite" number of measurements over the range of interest. The analyst's commendable dictum that "taken and found shall agree within acceptable limits" often leaves unanswered many questions about what goes on between the initial and final operation. In a broad sense, a properly designed recording system is a means for more complete understanding. From this point of view, it is improper to consider an automatically recording instrument as the delight of the lazy man, or as an iniquitous means of throwing a good man out of work.

Most recorders have been designed to record phenomena as a function of elapsed time. The new Leeds & Northrup Speedomax G $X-Y$ recorder enables one to record continuous and accurate curves of any two interdependent functions which can be converted into d.c. signals. The new recorder contains two speedomax electronic balancing systems, enabling one variable to be presented on a horizontal axis, the other on a vertical axis. The simplified diagram in Figure 1 shows how variable X, admitted to the input terminals in the form of a d.c. signal, controls the pen movement, and an appropriate signal representing variable Y determines the chart position.

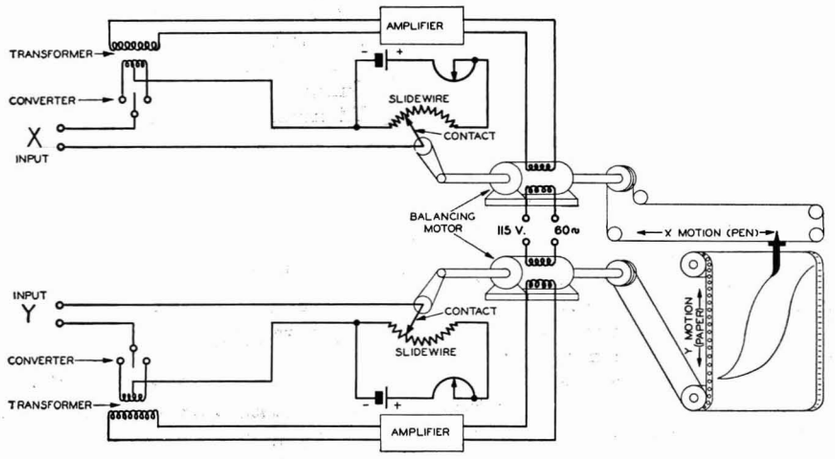


Figure 1. Simplified Diagram of X − Y Recorder

In the upper portion of the diagram, it can be seen that the input signal is chopped by a vibrator-converter element and appears as a.c. at the secondary terminals of the transformer. After considerable amplification, the a.c. signal is applied to one winding of the two-phase motor which drives the recorder pen in the appropriate direction. The motor shaft is also coupled to a circular slide-wire, the contactor of which is driven in such a direction that an e.m.f. is picked off to oppose the primary signal. When the exact value is reached, the input signal is canceled and the motor stops. The pen has now attained a position proportional to the impressed signal and has reached this condition under conventional potentiometric practice, although the operation has been automatic.

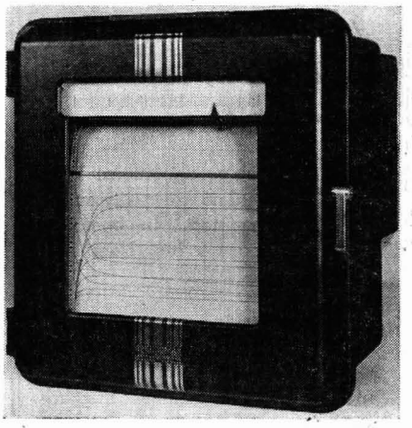


Figure 2. X − Y Recorder

An identical system operates on the signal representing variable Y, and the chart will move forward or backward in proportion to its direction and magnitude. The maximal excursion is 10 inches along either axis and therefore the curve may be accommodated anywhere within 100 square inches of chart space. The input for the X axis is 0 to 10 mv. or greater, although the lower range of 0 to 2.5 mv. is available on request. The range for the Y axis is 0 to 10 mv. or greater. The respective speeds of response for full scale deflection are 3 and 4 seconds. In both cases suitable capacitance-resistance networks are provided in the amplifiers to produce cor-

INSTRUMENTATION

rect damping to prevent oscillation or undue slowing of approach. The inputs can be derived from strain gages, thermocouples, phototubes, electronic circuits, or computer outputs.

A general view of the instrument is shown in Figure 2. A few typical applications are shown in Figure 3. The curve at the top shows the completely automatic thermal analysis of a clay sample. The ordinates represent the temperature and the abscissas the difference in temperature between the clay and some thermally indifferent substance. Three analytically significant inflections are apparent in this curve. A differential thermocouple supplied the X input, and the Y input was connected to a thermocouple which followed the slowly increasing furnace temperature.

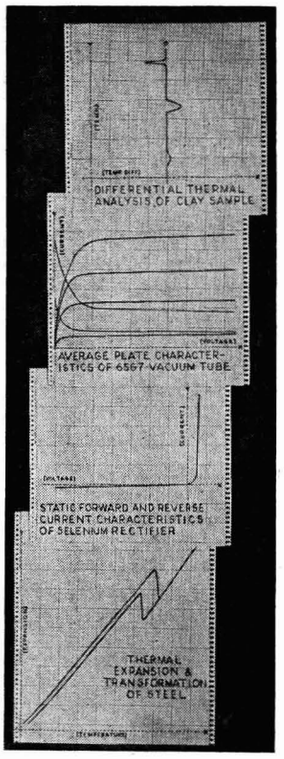


Figure 3. Typical Applications

The second chart illustrates the automatic recording of vacuum tube characteristics. In this example the plate current–plate voltage curves are drawn in for several fixed grid-cathode voltages, showing the typical behavior of a pentode.

The third curve illustrates the current-voltage curve of a selenium rectifier in the forward and reverse direction.

The fourth chart, which is drawn from a metallurgical application, more nearly

(*Continued on page 24 A*)

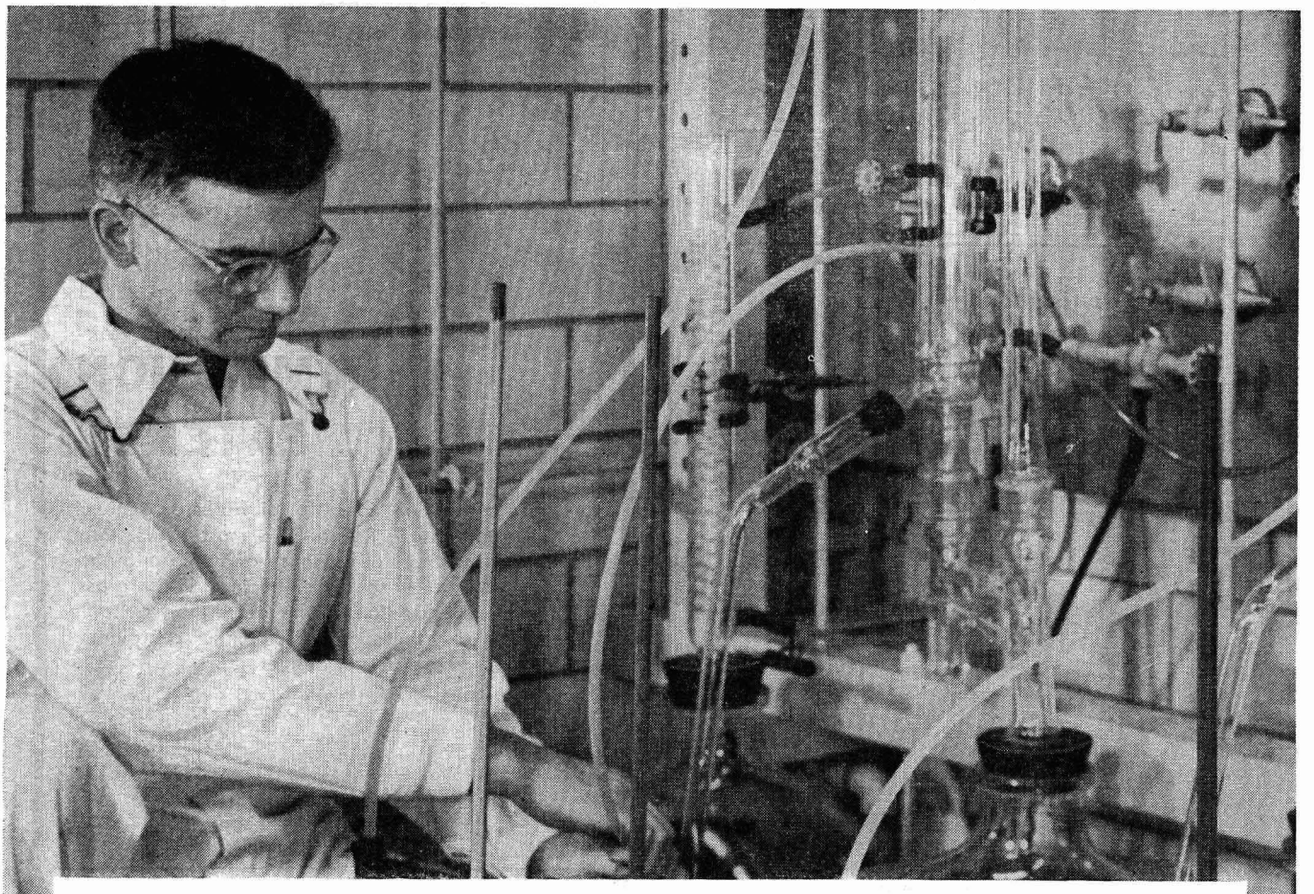
INSTRUMENTATION

illustrates the versatility of this instrument. Here the chart drive (Y axis) follows the expansion of a steel sample as a function of its temperature (X axis). The slope of the curve is a continuous indication of the coefficient of expansion and, in addition, the appearance of a definite phase transition is recorded. More significant, however, is the companion curve, obtained by allowing the furnace to cool slowly. This retraces the curve and exhibits the halt point during the phase transformation. Heretofore very elaborate systems have been required to adapt conventional recorders to the simultaneous delineation of the two variables.

From these few examples the analyst may see how readily a number of interrelated phenomena of analytical importance may be studied in intimate detail and without attention during the process.

Data Printers

Despite all the good arguments in behalf of automatic data recording, one may still ask whether the continuous recorder is the best solution for all cases. Frequently an automatic record will be examined and certain portions of it will be read off and reduced to numbers, for subsequent identification or computation. In such cases, it would be more convenient to print the numerical information directly. A wide range of equipment for this purpose is available, but its use has been confined almost exclusively to commerce and engineering. It is not our purpose to review alternative means of data recording, such as periodic photography of instrument panel banks, wire or magnetic tape recording, etc. We shall later describe briefly some representative printing equipment which has found wide and diversified use in industry and commerce. With little or no auxiliary equipment, these devices can find many uses in the research laboratory of the analyst. At the outset it should be emphasized that the real excuse for the use of data printers arises primarily from two situations. In the first, the value of data printing arises whenever values of one or more variables must be read in very rapid sequence. The chief difficulty here is what might be called the confusion element. The diametrically opposite case is afforded when an important measurement is to be followed over very long periods and constant personal attendance would be excessively burdensome. It is difficult for most of us to extrapolate sufficiently to realize the full potentialities of automatic data printing, but once the range and capabilities of existing equipment are understood it will be apparent that many new methods may be evolved with their aid.



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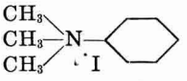
Chrysene (Eastman 4217) and *Anthracene* (Eastman 480-X) are useful, among other things, for catching culprits. Imperceptible dustings of chrysene fluoresce violet; ditto anthracene, but in another hue. An entomology prof we know of has for years had his own private mixture of the two, which he very, very lightly dusts over every field collection of specimens turned in by his students at the end of the term. The characteristic shade of blue-violet fluorescence excited by an ultraviolet lamp has trapped many a junior trying to get by with a senior fraternity brother's bugs. Have you any "bugs" in your business you want identified?

"Obsolete" to some...

$(C_5H_{11})_2C_6H_2$-1,4-$(OH)_2$

Di-tert.-amylhydroquinone (Eastman 3142) was an important chemical of commerce at one time. It was used to prevent discoloration and gum formation in kerosene on long overseas shipment. Nowadays there are other, better inhibitors. But what about the laboratory worker who wants a small quantity of a substituted hydroquinone? He *could* start writing letters to tonnage producers of petroleum additives. On the other hand, if he values his time, he just opens his Eastman Organic Chemicals catalog.

Cadmium-conscious...



Trimethylphenylammonium Iodide (Eastman 4423) is a quaternary ammonium salt we first prepared for a midwestern zinc firm back in the early thirties. Not long ago they told us there was no objection to revealing that they use it in a method they worked out for determining cadmium in their products, in concentrations ranging all the way from .006% to 90%. It seems to be a highly selective precipitant for cadmium in the presence of any amount of zinc. Zinc smelters generally are using it in their operations. It's also used to detect small amounts of cadmium in electrolytes and plant-waste liquors. If you're cadmium-conscious, we'll be glad to send you full details.

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NEW PRODUCTS FOR ANALYSTS

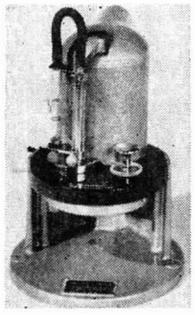
Equipment, Apparatus, Instruments, Reagents, Materials

Liquid Organic Silicates

Development of a new group of organic silicates which will remain liquid over an extremely wide temperature range has been announced by Oronite Chemical Co. These materials contain silicon—as do the already established and better known silicones—but have different chemical composition and properties. Some of Oronite's silicates are liquid over a temperature range of −100° to more than 700° F. Among their possible applications: heat transfer liquids, lubricants, surface coatings, and polishes. The fluids can be tailor-made to achieve maximum efficiency for each job. **1**

Windowless Flow Counter

Tracerlab is offering its SC–16 windowless flow counter, a low-background radiation counter designed for operation in either the Geiger or proportional regions. The instrument is essentially a shielded counter tube into which solid samples are directly inserted and through which a constant gas flow is maintained to prevent air contamination. The absence of even the thinnest window between the sample and the counting chamber makes this instrument particularly useful for the detection and measurement of alpha-radiation and of weak beta-radiation, such as that emitted by carbon 14 and sulfur 35. It also permits more efficient counting of low-activity radiation from very dilute samples.



A unique feature is the three-position rotating platform, which has three recesses for holding standard-size sample containers, such as planchets and brass rings and disks for filter paper. One position is for sample loading, one for preflushing, and the third for counting. The platform is advanced one position when a sample is to be changed. The measurement of the next sample can begin with no waiting time because all the air will already have been flushed out while the sample is located in the intermediate position. The end-on tube construction results in 2π geometry, which is the maximum possible geometrical efficiency for a flat sample. This fact, combined with the very low background (16 to 18 counts per minute) which in part is the result of the heavy lead shielding, further increases the sensitivity of this flow counter. **2**

Safety Goggles

Two completely revised and improved series of safety goggles—7000 and 3081 series—have been announced by the American Optical Co. The 7000 series metal safety goggles provide maximum eye protection, along with rugged construction, rigid saddle bridge, and reinforcing bar for extra strength. Temples now have brown tubing which cannot discolor and will outlast the life of the temple. A new feature allows the lenses to be replaced without removing side shields. The 3081 series has the same sturdy construction and the same new design features. Side shields of soft leather provide comfortable protection against heat and cold, flying particles, and light. Both goggles can be obtained with clear or Calobar and regular or 6-curve lenses. **3**

Double Monochromator

The double monochromator offered by the Central Research Laboratories dissects white light into narrow-band components of high spectral purity. It furnishes radiation in any portion of the spectral range from approximately 1400 A. in the ultraviolet to 25 microns in the infrared. This range is covered in several overlapping steps by means of interchangeable optical elements fabricated from glass, quartz, and various synthetic optical crystals. These optical elements consist of aspheric collimator lenses in prefocused interchangeable mountings and matched dispersing prisms of the same optical material as the lenses.

The entrance and exit slits are fixed in position, as are the

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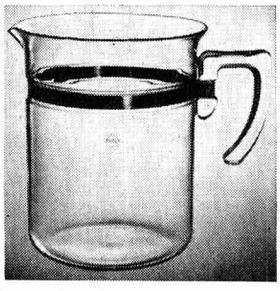
entrance and exit collimators, so that the aperture ratio remains constant at $f/4.5$ over the entire spectral range. Wavelength selection and focusing are accomplished simultaneously by the straight-line motion of a carriage carrying the curved center slit, by aligning the optical axes of the intermediate collimators with the light path, and by rotating the prisms to maintain minimum deviation. An electrically reversible synchronous motor drive is provided for wave-length control. Carriage speeds of 0.05, 0.10, 0.20, 0.50, 1.0, and 2.0 mm. per minute are obtainable.

Two general-purpose optical systems are available for this instrument: a light source mounting and motor-driven chopper assembly, utilizing a spherical mirror to give a half-size image of the source on the entrance slit; and a detector assembly with an adjustable ellipsoidal mirror forming a six times reduced image of the exit slit at a thermocouple or bolometer detector, and providing a mounting table for the experimental objects. Special external optical systems can be designed to meet particular requirements. The over-all dimensions of the instrument housing are: length, 25.5 inches; width, 15 inches; and height, 13.5 inches. **4**

Beaker with Handle

Requests for a better means of handling large beakers have been answered by Corning Glass Works, which announced a new 3000-ml. borosilicate glass beaker with a sturdy glass handle. Because the handle can be firmly gripped, the danger of dropping a beaker of hot liquid is minimized. The glass handle is attached by a stainless steel band, which also acts as a protective collar. Handles and bands are easily removed and are interchangeable. Chemically stable and resistant to thermal and physical shock, the handled beaker has a heavily beaded top rim to prevent chipping. **5**

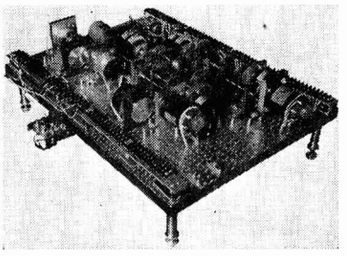


Stainless Steel Hose

Allied Metal Hose Co. has developed a new type of stainless steel hose for use where extreme temperatures, high corrosivity, or high internal pressures are involved. This flexible, corrugated metal hose is fabricated from thin-walled tubing and comes in sizes ranging from 0.25 through 4 inches in internal diameter. The wall thicknesses range from 0.010 inch in the 0.25-inch size to 0.035 inch in the 4-inch size. This type of hose is made in two patterns having either normal or close pitch. The normal pitch hose has a free flexing radius from 10 inches in the 0.25-inch size to 41 inches in the 4-inch size. The close pitch hose has a free flexing radius from 5 inches in the 0.25-inch size to 33 inches in the 4-inch size. **6**

Servoscope and Servoboard

Produced by the Servo Corp. of America, the Servoscope is a precision analyzer for measuring the response of servomechanism systems and components to sinusoidally modulated corrier and low-frequency a.c. signals. The Servoscope is effective over a continuously variable frequency range of 0.1 to 20 cycles per second. In conjunction with a cathode-ray oscilloscope, this instrument may be used to measure the relative amplitude and phase angle of any a.c. or d.c. servo system, servo component, automatic control, or regulator. Primarily developed for feedback control systems, the instrument may also be used as a stable generator of low frequency sine and square waves. A.c. carrier frequencies of 50 to 800 cycles per second are accepted to produce sinusoidally modulated suppressed carrier signals.



The Servoboard (see cut) is a set of standard precision mechanical parts, including gears, shafts, bearings, hangers, and mounting plates which, when coupled to the necessary motors, tachometers, synchros, potentiometers, and amplifiers, rapidly builds a flexible experimental mechanical assembly of a servo system, computer, or regulator. **7**

Determining Carbon in Steel

Savings up to 75% in the time required for making determinations of carbon in steel are claimed to be possible by the use of Fisher Scientific Co.'s induction carbon apparatus. A compact, self-contained unit replaces the furnace, rheostat, and external combustion train of conventional apparatus. The necessary external equipment consists of a cylinder of oxygen, a carbon dioxide absorption bulb, and a balance. With the new apparatus, carbon determinations can be made routinely in 2.5 minutes, as compared with the 10 minutes frequently required when other types of equipment are used.

The new apparatus employs a quartz sample holder and an induction-type coil which heats the sample with radio-frequency energy. Ignition takes place within 2 minutes. The

assembly includes a platinum-wire catalyst for converting carbon monoxide to carbon dioxide. The carbon dioxide formed by the ignition passes through an absorber which removes the sulfur dioxide present. The gas then passes through a carbon dioxide absorbent. Once the sample is weighed and placed in the quartz heating chamber, the combustion cycle is entirely automatic. The apparatus is designed to handle the full factor weight of 2.727 grams.

In a series of tests with National Bureau of Standards samples, it was found that the accuracy of the results was ±0.005%. The instrument will handle a wide variety of steel alloys with carbon contents ranging from 0.072 to 5.1%. It operates on 230 volts and 50- to 60-cycle a.c. **8**

Polyethylene Bottles

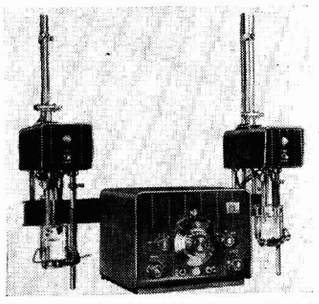
Designed for heavy-duty use, a 32-ounce unbreakable polyethylene bottle in the standard Boston round shape is now available from the Plax Corp. The new quart-size bottle is threaded to accommodate standard 38-mm., 430-finish acid pour-out caps. Thread finishes for other types of standard closures can be supplied on special order. The range of Boston round bottles offered by the company now comprises 1-, 2-, 4-, 8-, 16-, and 32-ounce sizes. **9**

Remote Pipet Control

The development and production of a remote pipet control have been announced by Nuclear Research and Development, Inc. Recognizing the need for a device which permits the remote pipetting of radioactive liquids, NRD has designed this instrument to perform all the necessary pipet operations without the necessity of handling the pipet at any stage of its use. The instrument can be used with pipets ranging from 100 λ up to 5 ml. It grasps the pipet and holds the lip in a tapered receptor. At the handle is a built-in 5-ml. hypodermic syringe. The sensitivity of the instrument may be increased by the use of a smaller syringe with an appropriate bushing. The device is made almost entirely of aluminum and weighs about 0.87 pound. **10**

Automatic Titrator

Beckman Instruments has announced the availability of a new automatic titrator. This instrument not only makes it possible to run accurate titrations more rapidly and conveniently than by manual methods, but it also gives objective, reproducible results that are free of errors caused by personal factors. In a series of titrations, it is only necessary to fill the buret, place the sample in the beaker, and turn the rest of the operation over to the titrator. Raising the beaker holder into position automatically starts the stirrer motor and begins the flow of titrating solution. A special electrical circuit anticipates the approaching end point and scales down the delivery of the titrating solution in progressively smaller increments to assure a highly accurate titration. When the end point is reached, the flow of titrating solution automatically stops and a light shows the completion of the titration—all without further attention from the operator. Only 1 to 1.5 minutes are required to complete many routine titrations, and even titrations to 0.1% accuracy can be completed in 2.5 minutes or less.



The adjustable holder accommodates beakers from 10 to 400 ml. in capacity, and the instrument may be used with all standard burets down to 5 ml. in size. As many as four delivery units can be accommodated by a single amplifier control unit, thus making a wide choice of titrating solutions immediately available. In addition to its use as an automatic titrator, the instrument can also serve as an a.c.-powered pH meter to give accurate readings over the range of 0 to 14 pH. It can also be used to provide voltage readings over the range of −600 to +1400 mv. **11**

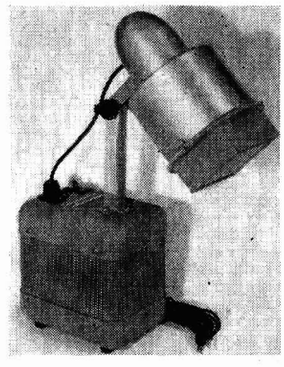
Density Measurement

A new direct-reading Densitrol has been added to Precision Thermometer and Instrument Co.'s line of liquid density measuring instruments. This instrument is of value not only where periodic checks of liquid density must be made but where process conditions, such as excessive corrosiveness or flammability, prevent the drawing of samples from the system. The instrument operates as a simple by-pass in any liquid line or vessel. Specific gravity may be read directly.

The instrument uses a totally submerged, chain-weighted plummet as the measuring element. The plummet is self-centering, operates without friction, and will not stick to the sides of the chamber. The reading scale is boldly marked and is read across a single reference line on the plummet. The length of the scale is 5 inches. Models are available with or without a thermometer, which is calibrated directly in terms of temperature correction and is mounted on the chamber. Suitable for high or low temperature or vacuum service, these instruments can be constructed from a wide variety of materials and are suitable for either clear or opaque liquids. **12**

Ultraviolet Light Source

The Hanovia Chemical and Manufacturing Co. offers a high-pressure quartz lamp as a source of ultraviolet light. The active length of the mercury arc is about 1.5 inches and it is C-shaped. The lamp is useful in microscopy, for absorption spectra work, for fluorescence analysis, and for illuminating optical apertures. A filter may be obtained which absorbs the short ultraviolet and visible radiations and transmits radiations largely at 3660 A. The lamp is connected to an ordinary 105- to 125-volt line through a 6-foot length of electrical wire. Approximately 100 watts are drawn by the burner. The ultraviolet intensity of radiations at 3130 A. and shorter, measured at 20-inch distance, is over 250 microwatts per square cm. About 3 minutes' time after starting is required for electrical stabilization and before the burner provides full ultraviolet intensity. **13**



Miniature Ion Exchange Unit

A new and refillable ion exchange unit delivers, from an ordinary faucet, water equal in chemical quality to the triple-distilled product. Called the Filtr-Ion and manufactured by La Motte Chemical Products Co., it is a small and low-cost application of the Monobed ion exchange principle, designed for small quantity uses where more elaborate equipment would be impractical. Although the tube is but 8.5 inches long and 1.625 inches in diameter, the Filtr-Ion unit is identical in chemical action with that of the huge Monobed water-conditioning apparatus commonly used in steam power plants.

The device is essentially a transparent tube filled with

Amberlite ion exchange resins. It is merely slipped over the end of a cold water faucet. Small holes in the top cap prevent excessive flow through the bed of resins. Deionized water is delivered through a small plastic tube. The unit is not intended to remove bacteria or impurities which are not ionized. As water passes through the apparatus, metallic and other ionic solids are taken out by a mixture of anion and cation exchangers. Fiberglas filters trap physical impurities.

The Amberlites change color when they become exhausted. Initially blue-black, the bed of resin turns light yellow in a gradually descending line as exhaustion progresses. When the yellow band reaches the bottom, the unit is refilled. Refill packages contain enough resin for two complete refills and include new filter elements as well. The quantity of deionized water delivered will vary with the hardness or mineral content of the raw water. Normally, up to 10 gallons of laboratory-quality water may be expected from each unit. **14**

MANUFACTURERS' LITERATURE

Toxicant. Folder describes Thanite, a toxicant used in space and residual sprays. A terpene chemical, it not only kills flies by direct spraying but also acts as repellent against the stable fly, horn fly, and housefly. As little as 1% Thanite in a 5% DDT household spray gives quick knockdown. Hercules Powder Co. **15**

Water Conditioner. Water conditioning equipment employing ion exchange is the subject of an 8-page folder. Included are descriptions of water softeners, hydrogen-zeolite water treatment, deionizing equipment, and general and specialized applications of ion exchange. Illinois Water Treatment Co. **16**

Alkyd Resin. A number of suggested paint formulations utilizing a new fast-drying glyptal alkyd resin are discussed in folder CDC-187. Glyptal imparts excellent adhesion, gloss and color retention, chemical and weather resistance, and flexibility to a variety of coatings. General Electric Co. **17**

Colloidal Graphite. Bulletin 427 describes the use of colloidal graphite as a parting compound. Because it is unaffected by temperatures up to 3500° F. in inert atmospheres, colloidal graphite is used to prevent sticking, corrosion, galling, and "freezing" of parts. Acheson Colloids Corp. **18**

Synthetic Tanning Agents. A company bulletin gives the history of naphthalene-type synthetic tanning agents, the mechanism of syntan chemistry, and the application of syntans in conjunction with chrome and vegetable tannages. The grades, analyses, and typical uses of synthetic tanning agents are mentioned. American Cyanamid Co. **19**

Refrigeration Oils. A 12-page booklet gives the characteristics and recommended applications for various grades of refrigeration oils. The "floc test" for determining an oil's suitability under low-temperature conditions is described. For various grades of oil, tables give technical data on viscosity, wax separation point, pour point, and dielectric strength. Sun Oil Co. **20**

Hormones. A list of hormones and their wholesale prices are set forth in data sheet. These hormones include diethylstilbestrol dipropionate, testosterone dipropionate, methyltestosterone, pregnenolone acetate, testosterone acetate, progesterone, and estrone. Mann Fine Chemicals, Inc. **21**

Synthetic Detergents. Technical Bulletin 50A-7 discusses dry mixing with various forms of Ultrawet, having average bulk densities between 0.16 and 0.37. Ultrawet SK is a spray-dried bead form of an alkyl aryl sulfonate, which is 35% active and 65% sodium sulfate. Ultrawet K, a flake detergent, contains a minimum of 85% alkyl aryl sulfonate; the balance, sodium sulfate. Atlantic Refining Co. **22**

***p*-Nitrophenyl Phosphate.** Bulletin 104 covers the detailed procedure for the use of *p*-nitrophenyl phosphate in the determination of both acid and alkaline serum phosphatase. This procedure is said to be an improvement over the usual methods in that it is faster and uses as little as 0.005 ml. of serum. Sigma Chemical Co. **23**

Cellular Rubber. A 20-page booklet describes the properties of cellular rubber: tensile strength, elongation, heat and sound insulation value, resistance to oils and chemicals, influence of heat and aging, toxicity, and others. Sponge Rubber Products Co. **24**

Instruments. An amply illustrated, 82-page booklet discusses instruments of value in research and analysis. Under the heading of analytical apparatus, the booklet gives details on robotized polarographs, titrators, and stills. Sections cover spectrometers, continuous gas analyzers, pyrometers, electronic indicators, high-vacuum gages, potentiometers, and other apparatus. Minneapolis-Honeywell Regulator Co. **25**

Publication. First issue of new magazine, *Labitems*, contains articles of technical and general interest to laboratory technicians, engineers, and management. Information about more than 50 products are included, among them balances, clamps, vapor pressure bombs, colorimeters, manometers, cartesian manostats, burets, titrimeters. Emil Greiner Co. **26**

Tackifiers. Tackifier resin emulsions for natural and neoprene rubber latices are the subject of data sheet A39. Also described are the compounding and handling of tackifier-latex adhesives. American Resinous Chemicals Corp. **27**

Electronic Parts. A new 130-page catalog of electronic parts provides full technical information on all items listed, in addition to illustrations and drawings of many components. Sun Radio and Electronics Co. **28**

Moisture Tester. A 6-page bulletin discusses midget moisture tester specifically designed for instantly determining the moisture content of wood, lumber, plaster, and wood products. The instrument measures moisture by an electrical resistance method. Readings in per cent moisture are taken directly at the touch of a button, with no conversion tables or long laboratory tests necessary. Tagliabue Instruments Division. **29**

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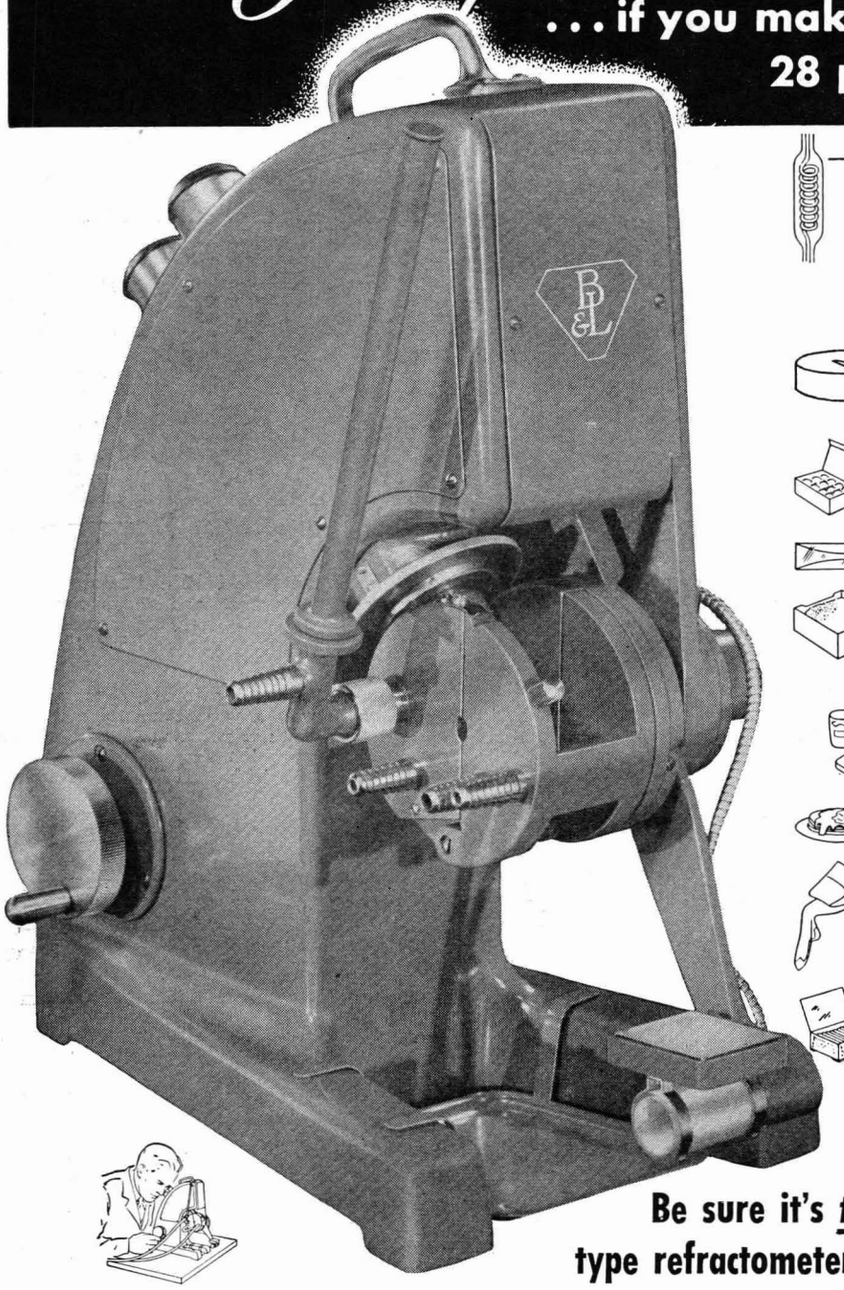

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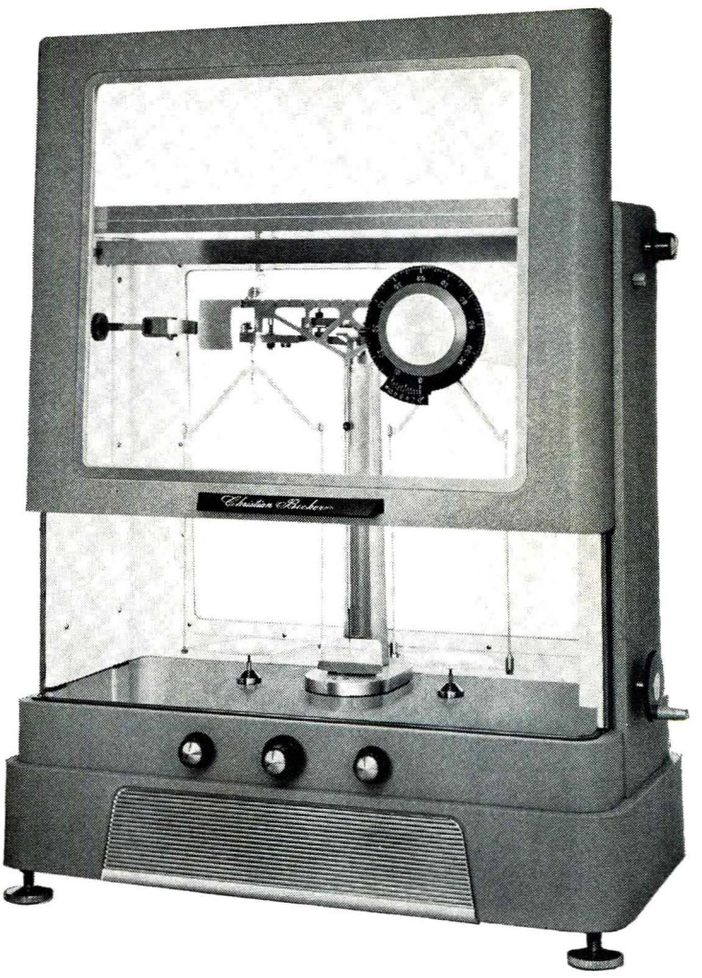

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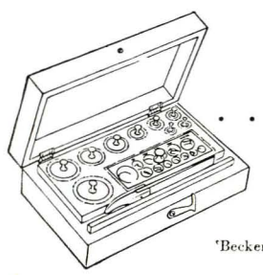

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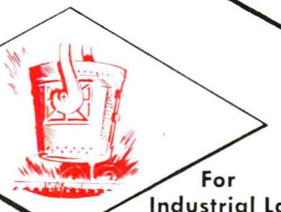

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