ANALYTICAL CHEMISTRY

AUGUST 1951

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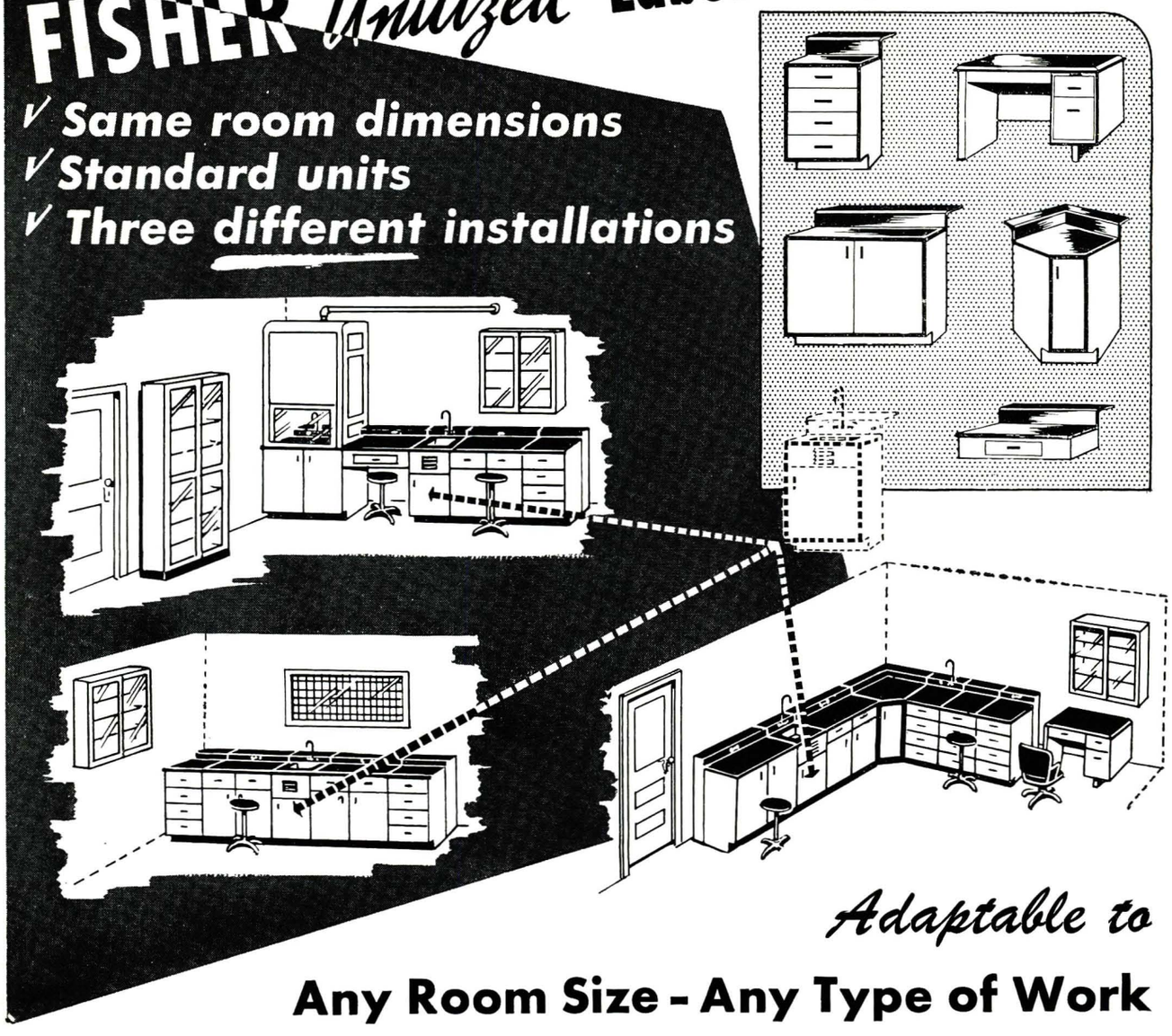

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ClO_3	<0.001 %	NO_3	<0.003 %
N. Comp. (as N)	0.0005 %	PO_4	0.0002%
SO_4	0.001 %	Ba	0.001 %
H.M. (as Pb)	0.0002%	Fe	0.0001%
Na	<0.02 %	Br.	<0.005 %
Ca. Mg. & NH_4OH ppt.			0.001 %
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Insoluble Matter	0.001	%
Neutrality	Passes A.C.S. Test	
Chlorate (ClO_3)	<0.001	%
Nitrate (NO_3)	<0.003	%
Nitrogen Compounds (as N)	0.0008	%
Phosphate (PO_4)	0.0001	%
Sulfate (SO_4)	0.0005	%
Barium (Ba)	0.0005	%
Calcium, Magnesium and NH_4OH Precipitate	0.005	%
Heavy Metals (as Pb)	0.0003	%
Iron (Fe)	0.0001	%
Sodium (Na)	<0.02	%
Bromide (Br)	<0.005	%
Iodide (I)	0.002	%

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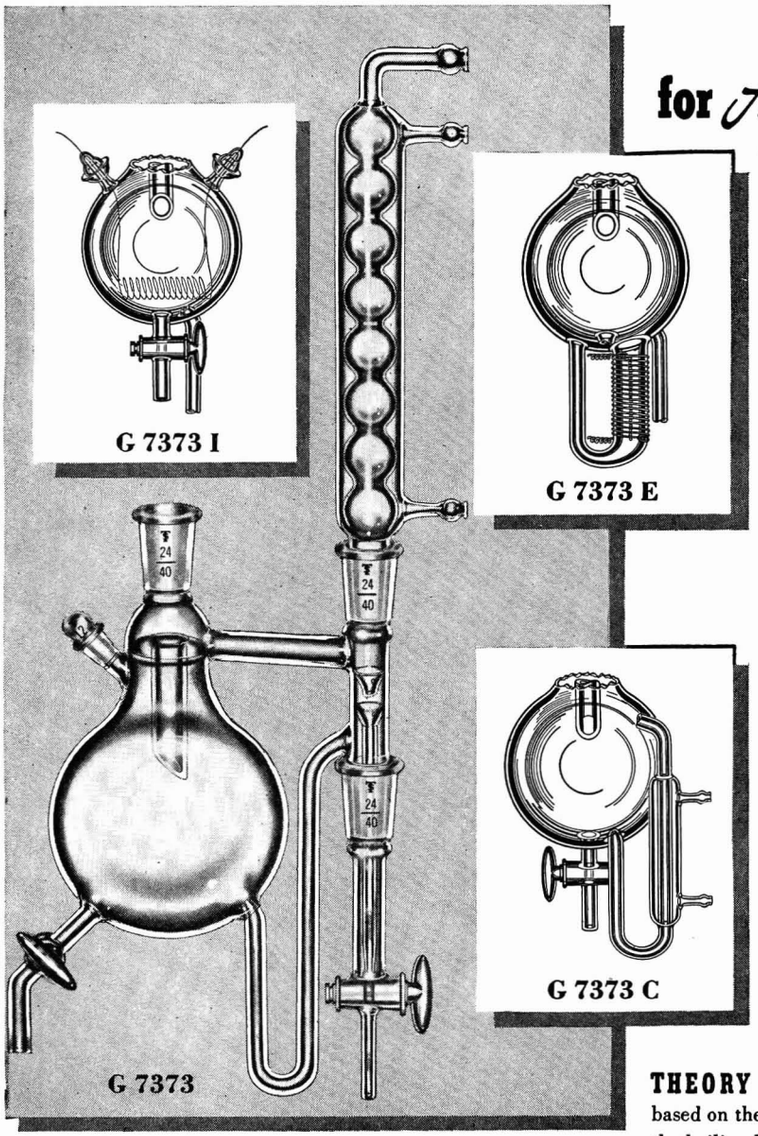

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*Othmer, D. F., *Anal. Chem.* **20,** 763 (1948)

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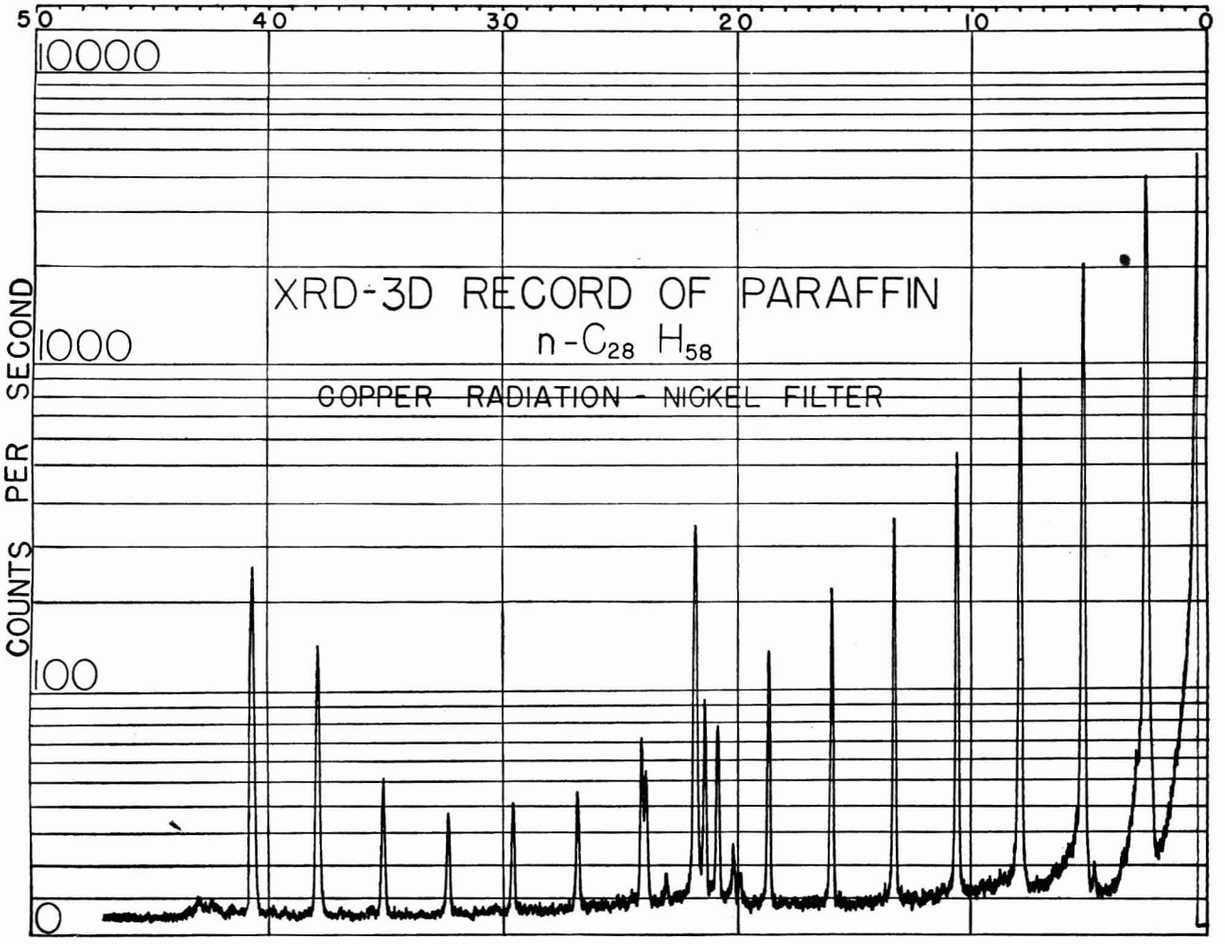

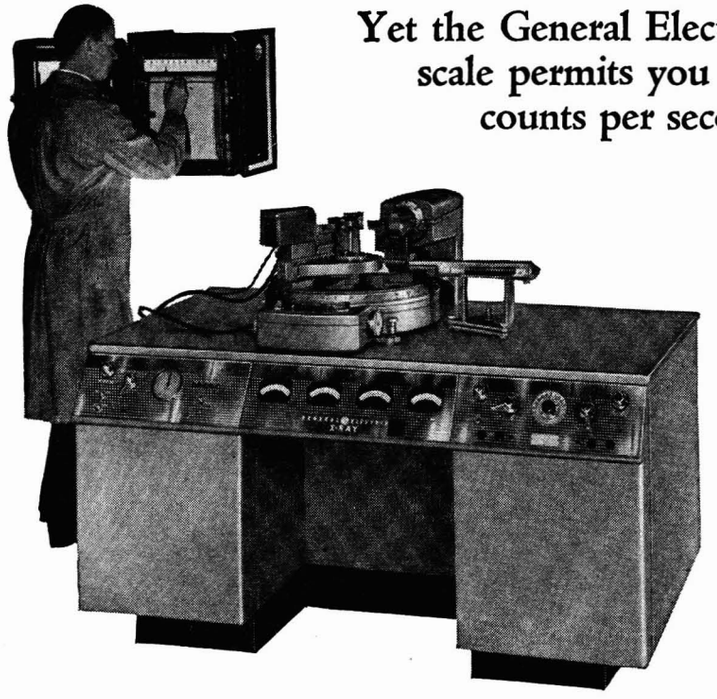

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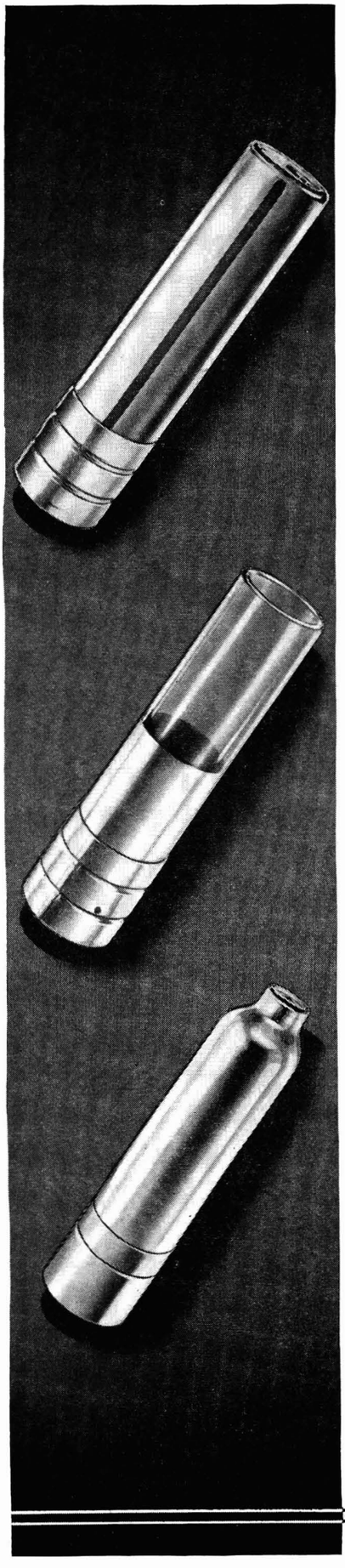

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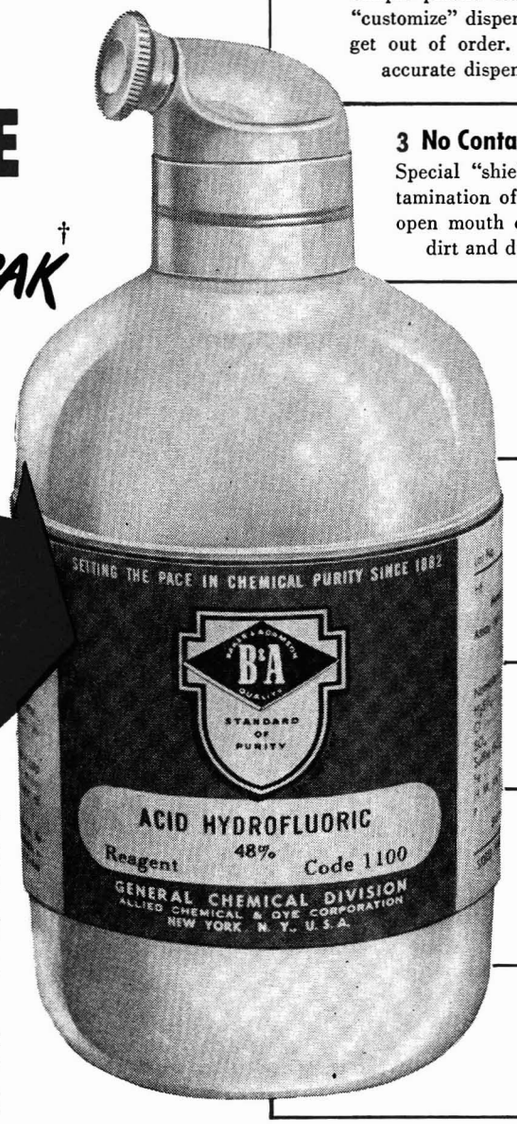

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Yet, in spite of all the fine accuracy built into these meters and couples, uncontrollable "cold-end" errors will frequently occur when so-called "compensating" lead wires are used. Here's why . . .

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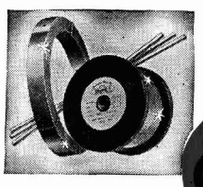
How to eliminate this common cause of "cold-end" errors? Simple! Use CHROMEL-ALUMEL EXTENSION LEADS with your CHROMEL-ALUMEL thermocouples. For only by using these wires of identical composition can you be sure of getting the full benefits of accuracy offered by today's improved pyrometers. Our Catalog 59-R tells the complete technical story . . . want a copy?

CHROMEL-ALUMEL couples and leads are available through your instrument manufacturer or pyrometer service company . . . ask for them by name!



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the analyst's column

THE final program of the World Chemical Conclave published in *Chemical & Engineering News* is a formidable one to digest. In this issue's editorial and this column, we turn the spotlight on that portion of interest to analysts. The ACS meeting in New York City, September 3 to 7, consists of symposia only, and of these seven are sponsored by the Analytical Division, one is jointly sponsored by the Analytical and Gas and Fuel Chemistry Divisions and one on sugar analytical methods is sponsored by the Sugar Division on Friday afternoon.

The XIIth International Congress, September 10 to 13, includes 50 original analytical papers for the four days.

Many famous analytical chemists will be visiting or giving papers at the symposia and at the congress along with those from this country. N. H. Furman, president of the ACS, has been chosen Domestic Honorary Chairman of the Analytical Section of the congress, while Fritz Feigl, well known for his research and publications on spot tests, is the Foreign Honorary Chairman. Included among the authors are the following from countries outside the United States: Fernando Blasco Lopez-Rubio, Jose de la Rubia Pacheco, F. Burriel-Marti, J. Ramirez-Munoz, and E. F. Caldas, Madrid, Spain; S. K. Susic and V. N. Njegovan, Zagreb, Yugoslavia; R. Belcher, A. J. Nutten, and W. I. Stephen, Birmingham, England; M. F. Kember, R. A. Wells, F. H. Burstall, A. F. Williams, and G. A. Wood, Teddington, England; G. Schwarzenbach, Zurich, Switzerland; Clement Duval, M. A. Kepes, M. Metayer, and A. Gross, Paris, France; K. Linderstrøm-Lang, Copenhagen, Denmark; A. Farag and H. A. Mangouri, Egypt; M. K. Zacherl, Vienna, Austria (editor of *Mikrochemie*); W. Schöniger and H. Lieb, Graz, Austria; and M. P. Ben-Yair and Joseph Jordan, Jerusalem, Israel.

We welcome these distinguished visitors on behalf of American analysts and trust that many of us will meet them and discuss mutual interests and exchange points of view during their stay with us. It is always pleasant to meet those whose contributions to a particular field have been outstanding, and our curiosity is aroused over the years as to the nature of the driving force and personality behind such successful research.

THE classic work of Pregl on organic microanalysis, and the first edition of his book, started a trend in the field of organic analysis which has practically displaced the

(*Continued on page 19 A*)

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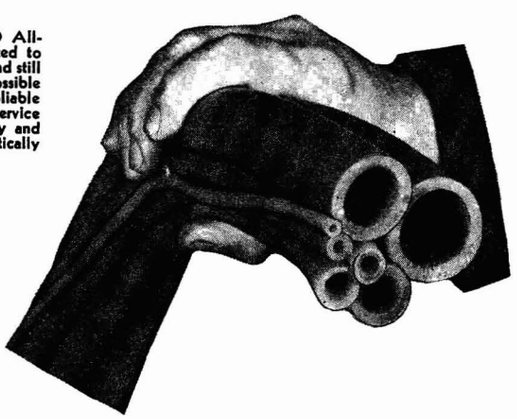

Size inches	I.D.	1/8	1/8	3/16	3/16	3/16	3/16	1/4	1/4	1/4
	Wall	1/32	1/16	1/16	3/32	1/8	3/16	1/16	3/32	1/8
Amber No. AC-36390 Price Per Foot		.08	.11	.12	.19	.22	.26	.13	.20	.25
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Size Inches	I.D.	1/4	5/16	5/16	3/8	3/8	1/2	5/8	3/4	1
	Wall	3/16	1/16	3/32	1/16	3/32	1/8	1/8	1/8	1/8
Amber No. AC-36390 Price per Foot		.40	.15	.23	.20	.26	.42	.55	.65	1.15
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cumbersome macroprocedures commonly used 25 years ago. The 5th English edition has been revised by Julius Grant and has just been released. We have watched for many years the European, English, and American developments in this field, and, while in this edition Pregl's original methods are generally given first choice, it is encouraging that other modifications are described and even recommended under certain conditions. We commend this feature. We quote from the preface:

It has been very gratifying to note that the new features then introduced (e.g., its international character, and references to physical methods used in organic microanalysis) have found favour with the reviewers and other users; these have been retained. One reviewer has deplored the gradual departure, in successive editions, from the original "Pregl," with its characteristic personal touch, which made almost unnecessary any references to the original literature.

I sympathize and agree with him. The original "Pregl" was, and always will be a classic, owing much to the personality of its author. However, as time passes and the ramifications of the subject spread both chemically and geographically, the influence of Pregl must inevitably weaken. The object of the present edition is, therefore, to provide an up-to-date textbook of the subject, embodying the tested experience of leading microanalysts all over the world, with full bibliographies containing references to original literature. I hope that the truly international character now imparted to Pregl will commend it to users in all countries; and that this and the other improvements noted will help to maintain its position as the standard work on organic microanalysis.

The report of C. O. Willits on standardization of microchemical methods and apparatus, given at the recent Analytical Symposium in Washington, was based upon a collaborative study by many laboratories of the non-Pregl modifications in use. It was encouraging to note that, in general, simpler and speedier elementary analyses are possible. They prove also that the microchemist is a conservative, because many of these modifications were first disclosed many years ago.

THIS issue and subsequent ones expand our Notes on Analytical Procedures section, so that the backlog of this important part of the journal can be published and these contributions may be kept on a more current basis. Some authors seem to feel that a Note contribution is considered less important by us. This is not so, and it is our goal to give them the same publication schedule as full length papers, because the ideas and conclusions disclosed by them are just as important to the development of analytical chemistry.

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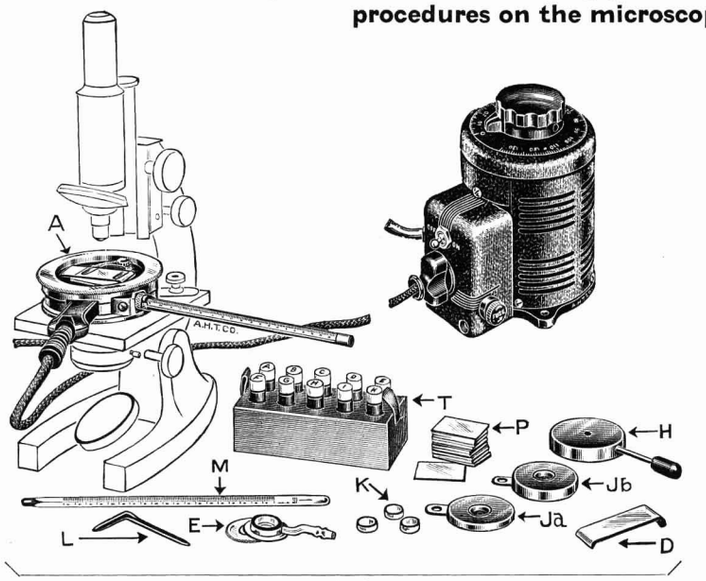

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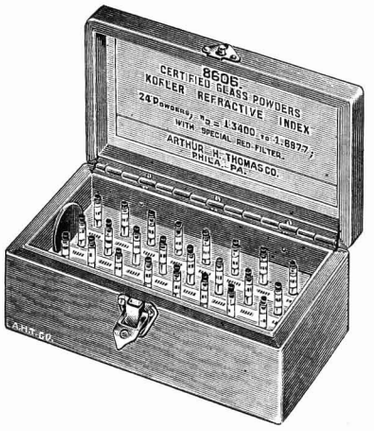

8606.

KOFLER MICRO HOT STAGE (Micro Melting Point Apparatus), A.H.T. Co. Specification, electrically heated and with stage calibrated thermometers.

For determining corrected micro melting points by means of a microscope with samples as small as a single crystal, permitting continuous observation of changes in the sample before, during and after melting.

Useful also for general micro-preparative work, sublimations, measurements of refractive indices at elevated temperatures, fusions, heating under controlled conditions, etc., and physicochemical studies. See Ludwig Kofler, *Mikrochemie, Vol. XV (1934), p. 242;* and L. Kofler and A. Kofler, *"Mikro-Methoden zur Kennzeichnung Organischer Stoffe und Stoffgemische" (Innsbruck, 1948).*

For temperatures up to 350°C, with an accuracy of ±0.5°C in the range to 200°C and of ±1.0°C in higher range. Can be used with transmitted, reflected or polarized light on any compound microscope providing magnifications from 50 to 100× with objective having working distance of 6 mm or more, and preferably with a metal stage.

The apparatus consists essentially of an insulated, chromium plated metal stage, 90 mm diameter × 20 mm high, heated by an embedded Nichrome unit, and with a central light well fitted with a condensing lens system. A threaded post takes either a fork for the micro slide or various sublimation blocks. A variable transformer, with stop to limit output to approximately 80 volts and dial usable only over range from 0 to 80 in single divisions, permits exact reproduction of settings.

The two thermometers, ranges +30 to 230°C and +60 to 350°C, respectively, have been calibrated on the individual Hot Stage with which they are to be used. A set of eight stable test reagents is included with each outfit. They are convenient, not only in acquainting the user with manipulation of the instrument, but also for the preparation of a calibration chart for the transformer settings.

6886-A. **Micro Hot Stage, Kofler,** as above described, complete assembly as shown in illustration, i.e., Hot Stage A with glass cover, two calibrated thermometers M, cooling block H, Fischer sublimation blocks Ja and Jb, glass baffle D, three sublimation dishes K, Kofler-Dernbach vacuum sublimation chamber E, fork lifter L, twenty-four micro slides P, set of test reagents T, and variable transformer but without microscope; in case, with detailed directions for use. For 115 volts, 50 or 60 cycles, single phase a.c. only.... **236.00**

6887-A. **Ditto, Hot Stage A,** only, with two calibrated thermometers M, cooling block H, glass baffle D and variable transformer, but without case or other accessories..... **164.50**

CERTIFIED REFRACTIVE INDEX POWDERS, Kofler. A set of glass powders and minerals of accurately determined average refractive index, in intervals of approximately 0.01, for the determination of the refractive indices of fusions of inorganic or organic substances, or of liquids, in the range of 1.3400 to 1.6877, incl. For example, fusions prepared on the Kofler Hot Stage are tested by the well-known "Becke-Line" method in accordance with procedures developed by Dr. L. Kofler and his co-workers.

8606. **Certified Refractive Index Powders, Kofler,** as above described, range 1.3400 to 1.6877, set of twenty-four powders, each in glass tube containing sufficient for 200 tests, complete in wooden case with red glass filter and detailed directions for use..... **26.50**

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ANALYTICAL CHEMISTRY

Walter J. Murphy, Editor

The World Chemical Conclave

THE Diamond Jubilee Meeting of the AMERICAN CHEMICAL SOCIETY, September 3 to 7, and the International Congress of Pure and Applied Chemistry, September 10 to 13, in New York have many attractions for the analytical chemist.

The program of the Division of Analytical Chemistry of the ACS is nicely balanced between theory and practice. Only symposia will be presented at the anniversary meeting. The Division of Analytical Chemistry will conduct seven symposia and cosponsor another with the Division of Gas and Fuel Chemistry.

Absorption and Emission of Radiant Energy
Nucleonics and Tracer Techniques in Analytical Chemistry
Economic Aspects of Chemical Analysis in Manufacturing
Polarographic Methods
Microchemistry
Chemical Kinetics and Mechanisms of Analytical Reactions
Physical and Chemical Equilibria
Modern Techniques in Research on Coal and Related Products (cosponsored)

This is a program to whet the appetite of any analytical chemist. A great deal of thought and planning obviously have been done by the officers and Executive Committee of the division. Indeed, the original plans were drawn up at the Houston meeting in the spring of 1950 during the chairmanship of Grant Wernimont. H. H. Willard, the present chairman, Secretary William G. Batt, and the chairmen of the symposia—Brode, Clarke, Duke, Huffman, Lingane, Rodden, and Swift—have developed the most outstanding program in the history of the division. Of very special significance is the number of foreign analytical chemists who are presenting papers in the symposia.

The dinner of the division will be held on Tuesday evening, September 4, and the speaker, C. J. Van Niewenburg of The Technical University at Delft, Holland, needs no introduction to our readers.

The program of Section 2, Analytical Chemistry, of the congress lists an even 50 papers necessitating sessions Monday, September 10, through Thursday, September 13. Again we are pleased to note how many papers will be given by foreign analysts.

The formal scientific programs of the Diamond Jubilee Meeting of the Society and the congress are by no means the only attractions. A wide variety of plant trips have been arranged during the congress, part of the "World Chemical Conclave." Many will be of special interest to the analyst. The social side has not been neglected. Those attending will find much to do and to see in New York. But most important, the coming meetings will provide us with an opportunity to meet personally many of the leaders in analytical chemistry in other parts of the world.

The 16th conference of the International Union of Pure and Applied Chemistry (the third event constituting the World Chemical Conclave) will be of considerable interest to analysts, even though the number participating in its deliberations will not be great. Several of the commissions of the union deal with fields directly connected with analytical chemistry. The importance of analytical chemistry was fully recognized and acknowledged at the Amsterdam meeting two years ago, when the sections of the union were revised. At that time a section on analytical chemistry was established. The sections as now constituted represent the six principal branches of chemistry.

Lastly, there is the Washington session of the International Union, September 14 and 15. On the evening of September 14, a joint banquet of the International Union and the National Bureau of Standards will be held under the auspices of the National Academy of Sciences to celebrate the 50th anniversary of the establishment of the Bureau of Standards.

Two very strenuous but intensely interesting and profitable weeks are just ahead for many analysts. We hope you will be among those present.

Speakers on Analytical Subjects

WE have just received a copy of the list of speakers prepared by the Speakers Procurement Committee of the Division of Analytical Chemistry and we extend our congratulations to B. J. Heinrich, chairman of the committee, and his associates for an excellent piece of work.

The list, published on page 1190 of this issue, contains 27 specific subject classifications and also a list of miscellaneous topics.

Just about every subject of possible interest to analytical chemists is covered. Other divisions of the Society would do well to follow the leadership of the Division of Analytical Chemistry. Copies of the list can be obtained from B. J. Heinrich, Phillips Petroleum Co., Bartlesville, Okla.

Methods for the Determination of Water

Presented before the Division of Analytical Chemistry at the 118th Meeting of the American Chemical Society, Chicago, Ill.

The determination of water is one of the most common that the analytical chemist is required to make. Water is used in the preparation of many materials and remains present in the product as a combined constituent or as a contaminant or diluent. Many materials pick up water readily from the air. In many cases the water present functions only to dilute the active ingredient, but a knowledge of its amount is important because the activity of the main constituent depends upon the water content. Many compounds are bought and sold on a "dry" or "water-free" basis. The determination of water, therefore, must precede other determinations on a sample.

It seems logical that a symposium should be organized to consider some of the ways in which water can be determined. This program was planned to discuss four important methods for determining water, with full realization that not all methods have been covered. The simplest and probably the oldest approach is by drying in an oven and determining the loss in weight. Another method used for a long time is that of distilling the water from a sample with the aid of another immiscible liquid and measuring the volume of the separated water. More recently, the Karl Fischer reagent for water has found wide acceptance. In this case a specific reaction occurs in which the water takes part, and not only can water as such be determined but other reactions in which water is taken up or given off can be followed through the water determination. The fourth method is based on an electrical technique for determining water. Instruments which measure resistance or conductivity have been adapted to the determination of water and provide a precise method for the purpose.

J. W. STILLMAN, *Symposium Chairman*,
E. I. du Pont de Nemours & Co., Inc.,
Wilmington, Del.

Methods for Determination of Moisture

Oven Drying

C. O. WILLITS

Eastern Regional Research Laboratory, Philadelphia 18, Pa.

THE determination of moisture in organic materials, whether they be synthetics or biological materials, is not a simple procedure. Even today a common concept of moisture analysis is that implied by an old (1885) procedure of the Association of Official Agricultural Chemists, which specified moisture as the loss in weight occurring when a substance was heated at 98° to 100° C. This simple method will give approximate moisture values, but cannot be depended upon to produce accurate results because it fails to recognize the complexity of the process of water removal. The results obtained by the time-honored thermal drying methods may sometimes be wrong, but nevertheless oven methods for moisture analysis generally are the standard for most other moisture methods, whether by distillation, electric moisture testers, chemical reactions, or some other physical means.

During the heating of a moist organic substance the following changes may be expected to occur: volatilization of water as moisture, volatilization of other adsorbed material, and volatilization of the gaseous products formed by nonreversible decomposition reactions, such as carbon dioxide, carbon monoxide, methane, hydrogen, and water. This decomposition does not begin at any particular temperature, but goes on at all temperatures at widely different rates.

Nelson and Hulett (*5*) in 1920 found that when an organic substance was heated at constant temperature the amount of water liberated from that existing as the external phase was dependent upon the temperature and that the rate of removal of this water, liberated by heat at constant temperature, is rapid at first, then falls off, and, in time, ceases. They plotted the per cent moisture against time and obtained isotherms (Figure 1) for successively higher temperatures. When a curve is parallel to the time axis,

it means that no more water is being liberated at that temperature. These curves indicate that the water of the external phase remaining after each heating temperature has such a low vapor pressure that no further loss of water occurs. This low vapor pressure, according to Nelson and Hulett, may be due to successively thinner layers of water, or to other physical phenomena such as capillary attraction and diffusion.

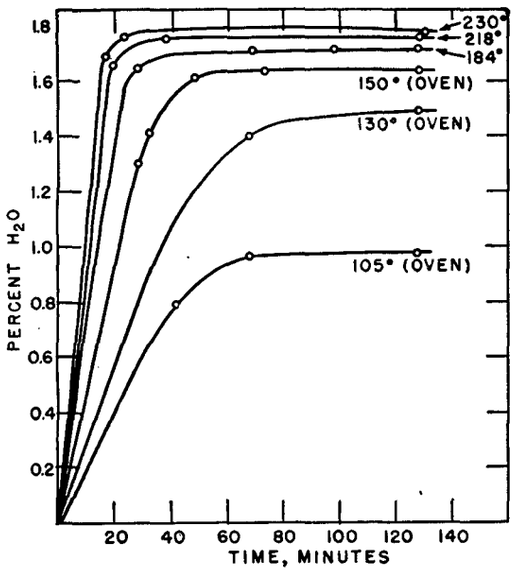

Figure 1. Removal of Water at Constant Temperature

What then is the true moisture, if the one obtained by the 100° C. drying is not an end value, and which curve shows the beginning of losses due to decomposition of the sample? Porter and Willits (*7*) obtained a similar family of drying isotherms for starch and suggested a means of estimating the temperature at which losses in weight caused by decomposition predominate. If the amount of water liberated, or the loss in weight, is plotted against temperature, the curve shows a distinct break (Figure 2). The first curve indicates loss of external phase water; the second, losses due to volatilization of decomposition products plus water.

A more nearly reproducible method would specify a temperature that would lie on the flattest portion of a moisture-temperature curve. It is unfortunate that organic substances cannot be heated to such a high temperature that all the water molecules must be evolved as a gas. It has been suggested that the lower member of the loss in weight-temperature curve be extrapolated to the critical temperature of water and that the height of this point (*5*) on the loss-in-weight axis would be the value of the true moisture content of the sample. Unfortunately this cannot be done, as the curve is not a straight line, and because of its changing function it is nearly impossible to establish a mathematical expression for the curve. Therefore, although an absolute moisture value cannot be expected by oven drying methods, it is possible to obtain a value that is relatively close to this and is reproducible, by using a temperature indicated by the flattest portion of the curve and a time sufficiently long to ensure reaching constant weight.

Many investigators have contended that naturally occurring organic substances such as cereals and other plant tissue could not be dried without decomposition of the original moisture-free substance. The foregoing method does not conclusively prove that the dried substance is the unaltered original substance minus its external phase, water.

Several attempts have been made to show that drying can occur without decomposition of the substance.

In the case of starch, Porter and Willits observed a light brown discoloration, indicating an alteration in some of the samples during the time necessary to establish the constancy of weight at 180° C. However, they noted no change in the reflectance or in the solubility of the starch until the constant-weight period was reached. While changes in color and solubility were then indicated, they did not affect the constancy of weight until after nearly 4 hours, when a drastic breakdown or alteration occurred, accompanied by a further loss in weight. Sair and Fetzer (*9*) measured thermal decomposition by determining "reversibility moisture." It has been known for a long time that completely dehydrated biological products exhibit a hysteresis effect on resorption to the original moisture level. Urquhart and Eckersall (*10*) and Pidgeon and Maass (*6*) have shown that cellulose which contains 1% of moisture loses it reversibly. Mellon and coworkers (*4*) have shown similar results for proteins with water contents exceeding 15%. In many biological materials it is relatively easy to remove moisture down to the 1% level, but to remove the final water is difficult. A large number of methods have been proposed to accomplish this. The one most often used is vacuum oven drying, but it is not known what occurs during heating, even though it may be in vacuo. Is externally held water the only volatile given off?

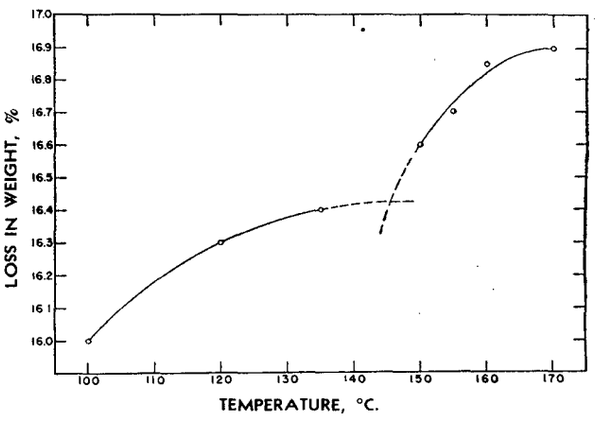

Figure 2. Loss in Weight of Potato Starch Dried at Various Temperatures (*7*)

Sair and Fetzer (*9*) applied the technique of reversible drying to cereals to determine whether or not decomposition occurs, or if the original water present in a sample can be accounted for. Using ground corn, a sample was dried at 40° C. for 340 hours, a period well beyond that necessary to attain constant weight. As the weight remained constant, it was considered that the corn was anhydrous. Another sample of the same ground corn was heated at 100° for different lengths of time.

As they assumed that drying at 40° C. for 340 hours resulted in an unaltered, perfectly anhydrous material, why go further?

Determination of moisture by oven drying is of great interest, and has been the subject of extensive research and many papers. This resume correlates these studies and the theories and methods of thermal drying, particularly for organic and biological materials. The important factor in thermal drying is the differential between the vapor pressure of the substance to be dried and the vapor pressure of the atmosphere of the drying chamber. A favorable differential can best be attained by use of chemical drying agents rather than by vacuum. The temperature employed should be as high as possible, without causing appreciable thermal decomposition. Guides are given for establishing thermal drying conditions of most organic and biological materials.

The answer is simple. A drying period of almost 2 weeks would be impractical in routine analysis and a shorter method utilizing higher temperatures must be used. Their 2-hour drying period, at 100° C. in a vacuum oven, indicated no irreversible changes. However, after 4 hours of heating, the sample on resorption did not have the same dry weight as the control. They considered that two processes occurred, a change in adsorption capacity, and decomposition.

Makower and others (*3*) also attempted to establish empirical conditions of time *vs.* temperature, whereby the true moisture could be measured. In work with dehydrated vegetables they became interested in the problem because of the discordant moisture results obtainable on similar samples. The basis of their technique assumes that samples of onions and cabbage dried in vacuo at 60° C. for at least 60 hours become essentially dry. By their procedure such a sample was dried at 60° C. for 100 hours and weighed at intervals to obtain a moisture curve. A known weight of water was then added, and after equilibration was complete, the wetted sample was redried at 60° and weight losses were measured at frequent intervals. The time at which the loss in weight equaled the amount of added water was noted as indicating the time required for drying the original moist sample. This is true only if the two curves are similar.

Makower also had to make three assumptions. The first was that essentially all of the water was removed at the end of the first drying run. This was assumed to be correct moisture, because the long drying period yielded weight loss of only 0.005% per hour and redrying time remained unchanged even when the first drying run was conducted for 40 to 50 hours longer. The second assumption was that weight changes (loss) increased with heating time in the same manner for both drying runs. The third assumption was that a negligible amount of decomposition occurred in drying the sample from higher to lower initial moisture levels. This was taken to be valid because the time of drying was relatively short and temperatures were below those at which appreciable decomposition occurs. The moisture content of the original sample was thus equal to the algebraic sum of the known moisture content of the remoistened sample and the shift along the weight-loss axis found in testing for similarity of the two curves.

THEORY

Moisture may occur in a sample in several ways. The ones of interest here are moisture occurring principally as an external phase, and moisture occurring as one or more molecular layers plus liquid water. The rapidity with which moisture can be distilled from the surface of a solid phase is a function of the water vapor pressures and of the drying temperature.

Recent practice has been to remove moisture from a solid at the lowest possible temperatures. All that is really required is that the partial vapor pressure of water in the gas phase (air) be lower than the vapor pressure of the water in the sample. Thus we can have thermal drying at temperatures below the freezing point of water, as in the case of lyophilization, or at higher temperatures as long as the applied energy (heat) is insufficient to cause noticeable decomposition of the solid undergoing drying. However, at higher temperatures an advantage is gained because the vapor pressure rises rapidly with increase in temperature. Thus, potato starch containing 16% moisture has a water vapor pressure of only 12 mm. at 25° C., but this is raised to 539 mm. at 92° C. Some observers have noted that the temperature effect is even greater when only very small amounts of water are present. Thus, Makower observed that in drying potatoes at 70° C. a difference of only 1° C. produced a change of 0.1% in the moisture.

Katz (*2*) presented the relationship between the water vapor pressure in the air and the water content of macromolecular substances such as cellulose, casein, starch, and gelatin. A similar relationship was shown by Mellon *et al.* in the water sorption of proteins. The shape of the absorption curve indicates that relatively large amounts of water can be held by these substances at very low water vapor pressures. This would mean that, depending upon the nature of the slope of the curve at high temperatures, changes in the water vapor pressure of the air (or changes in relative humidity) have a great or a small effect upon the water content of the solid being dried.

The vapor pressure of liquid water is not very sensitive to changes in the pressure of the atmosphere. At ordinary temperatures an increase of 1 atmosphere pressure increases the vapor pressure of water by only 0.1%; the fractional increase is less the higher the temperature. It is to be assumed, therefore, that the influence of 1 atmosphere of excess pressure on the vapor pressure of adsorbed water on a solid surface would likewise be negligibly small. The ultimate moisture content to which a solid is reduced by thermal drying should be the same for the same partial pressure of water vapor, regardless of the atmospheric pressure.

The partial pressure of water vapor in air does not vary with temperature. Drying rates depend on the difference between the vapor pressure of the external phase of a solid being dried and the vapor pressure in the air. The increase in the rate of drying due to rise of temperature is not due to the lowering of the vapor pressure in the air, but rather to the increase in the vapor pressure of the water on the solid. The rate of drying can be further accelerated by increasing this water vapor pressure differential through lowering the vapor pressure in the air. This can be accomplished by use of a desiccant or a vacuum.

Assuming that the absolute pressure of water in the air surrounding the sample is the important factor, the benefits of a vacuum are real only when the amount of moisture remaining on the sample becomes so small that its vapor pressure will not influence the vapor pressure of the vacuum chamber air. In practice seldom are vacuum ovens used in moisture analyses operating below 1 mm. of mercury pressure. A much simpler procedure to obtain a low vapor pressure is to diminish the water vapor pressure in the air of an oven by utilizing a chemical drying agent. Table I shows that a number of such drying chemicals (*1*) can reduce the water vapor pressure far below that which is easily attainable with the usual vacuum oven.

In using a drying agent in the determination of moisture, it has been customary to enclose the sample and the desiccant in the same container, so that the moisture in the atmosphere will not be introduced to the system. Having the desiccant near the sample reduces diffusion time, with gained efficiency. With these facts accepted, it was easy to proceed a step farther and combine the good effects gained from the use of both a vacuum oven and a desiccant. This has been applied extensively in Abderhalden and pistol-type dryers, but seldom in larger vacuum ovens.

If no air were let into the vacuum oven, the pressure of the water vapor in the oven would rise to a value that would make the usefulness of the vacuum oven questionable, especially for samples of high moisture content. There will come a time when the efficiency of the oven will be limited by the rate of water diffusion into the pump. To compensate for a low diffusion rate, a small amount of air is let into the oven to produce a continuous sweeping of the oven chamber. This will not lower the water vapor pressure in the oven. A low vapor pressure in the oven can be obtained if the air is desiccated before it is let into the oven, a scheme most often used in large vacuum ovens. Actually, with the use of predesiccated air an air oven would be as effective as a vacuum oven.

SOURCES OF ERROR IN THERMAL DRYING

Some of the sources of error in thermal drying were reviewed by Reith *et al.* (*8*).

Nonwater Components. In thermal drying, errors will be introduced if the material being dried contains any substances in addition to water which have an appreciable vapor pressure under the conditions of the drying. These will cause an additional loss in weight of the sample aside from the loss due to water, and because the two are not readily distinguishable, they will cause high "moisture" values.

Chemically Bonded Water. Small quantities of water may be lost through chemical reaction induced by heat as in the case of dextrin formation, inversion of disaccharides, or hydrolysis of proteins.

Nonwater Solvents. Often in the case of preparative organic substances which have been crystallized from such solvents as

alcohol or acetone, moisture (loss in weight) values are too low as indicated by elemental analyses. The incomplete "drying" is caused by these solvents being held by the substances throughout the thermal treatment.

Autoxidation. Chemical changes cause an apparent low moisture due to increased weight of sample by autoxidation, especially in fats and oils.

Decomposition. When the temperature of drying is sufficient to cause thermal decomposition of the substance and the products of this decomposition are volatile, high moisture will result. Errors of this sort are most common with organic substances, but inorganic carbonates, for example, can also decompose.

Most of these errors can readily be avoided, with the possible exception of those caused by nonwater volatile constituents. Three methods that tend to minimize errors due to nonwater volatile constituents are: collection of the volatilized water as ice, use of an absorbent specific to water, and the indirect methods of Makower and Porter.

Table I. Relative Efficiencies of Drying Agents

(Values of residual H_2O per liter of gas dried at 25° C.)

Drying Agent	H_2O, Mg.	Drying Agent	H_2O, Mg.
Filter at −194° C.	1.6×10^{-23}	$CaBr_2$	0.2
P_2O_5	2×10^{-5}	CaO	0.2
$Mg(ClO_4)_2$	5×10^{-4}	$CaCl_2$ (granular)	0.14 to 0.25
$Mg(ClO_4)_2.3H_2O$	2×10^{-3}	H_2SO_4, 95.1%	0.3
KOH (fused)	0.002	$CaCl_2$ (fused)	0.36
Al_2O_3	0.003	$ZnCl_2$	0.8
H_2SO_4	0.003	$ZnBr_2$	1.1
MgO	0.008	$CuSO_4$	1.4
NaOH (fused)	0.16		

Losses of water through chemical reaction in dextrinization or protein hydrolysis occur principally under conditions of high moisture and elevated temperatues. This type of error can best be prevented by drying the sample at low temperatures until the bulk of the water has been removed and the possibility of the reaction reduced to a minimum. The substance can then be dried at the desired elevated temperature. This is the accepted practice in Europe for determination of moisture in starch.

When incomplete removal of moisture is suspected from substances previously treated with such solvents as alcohol or acetone, it is likely that these solvents, and not water, have resisted volatilization by the thermal drying method. Increase of temperature or change in the pressure of the vapor through vacuum or other means is usually ineffectual. The best remedy is to humidify the sample, in which process the solvent is replaced by water and then this is removed by the accepted thermal method.

To prevent errors from oxidative changes, the simplest method is to use an oxygen-free, inert gas—nitrogen or carbon dioxide—in the thermal drying chamber.

For substances that undergo thermal decomposition there is usually a critical temperature at which this occurs at sufficient rate to introduce errors in the moisture values. In many instances it is possible to determine the critical temperature of decomposition of a substance and, knowing this, to use temperatures in a range below this value for thermal drying.

In thermal drying there are additional sources of error. One of these is slowness in establishing equilibrium between the vapor pressure of the water of the solid and that of the atmosphere. Because we are primarily concerned with absolute dryness, it is not necessary to discuss the effect of exposure of substances to atmospheres of different relative humidities and the time required for equilibration. However, as a substance is being dried and the moisture content approaches zero, the vapor pressure and consequently, the rate of volatilization of the water become very slow. The resulting changes in weight of the substance or weight of water absorbed are so small that the conditions of constancy are assumed to have been fulfilled. Actually, the substance may still contain measurable quantities of water, as in the case of lactose hydrate or starch. This error can be avoided only by drying for a longer time, using a higher drying temperature, or both.

The formation of a crust which is impervious to moisture has always been a source of error. In the moisture analysis of high sugar samples, sirups, and the like, by thermal drying, a water-impervious crust almost always forms, causing nearly complete stoppage of evaporation of the remaining moisture. This error can be eliminated by use of sand dishes to increase the exposed surface or by top drying at moderate temperatures under infrared heat lamps.

Many plant materials tend to crust before drying is completed, making it difficult or impossible to remove all the moisture. This cause of error can be partially eliminated by drying first at low temperature to remove most of the water without crust formation, and completing the drying at the requisite elevated temperature.

The physical structure of plant or animal tissue may also contribute to errors in drying. Both sorts of tissue often contain as high as 60 to 90% moisture. The removal of moisture from the cells, vacuoles, and tubes of these tissues presents separate problems. Cell moisture must diffuse through the cell wall before it can be volatilized. This becomes increasingly difficult as the drying proceeds. The moisture leaves the surface of the cells by diffusion and if the material is thick it requires considerable time for this to take place. Furthermore, as the cells lose water and the cell fluid becomes more concentrated, there is a lowering of the vapor pressure of the residual water. To drive the last traces of moisture out of the cells at a reasonable rate, the temperature must be increased, and this often makes the cell walls less permeable to water. Heat also tends to seal the tubes in the tissue, making diffusion of deep-seated water vapor very slow. Often the surface of plant parts, such as stems and twigs, is almost impervious to moisture and all the water has to diffuse out longitudinally. These errors due to physical structure can often be avoided by fine grinding to diminish the distance through which the water vapor must diffuse, and by using multiple stage drying, so that most of the moisture can be removed at temperatures at which the cell walls remain permeable to water.

A common source of error in moisture analysis is in sampling, particularly for substances with high moisture content. These samples usually have water vapor pressures much higher than the ambient air, and therefore undergo rapid changes. A typical example is freshly cut leaves. With such material having a water content of 90% or more, an error of sampling which accounts for only a 1% error in moisture can cause a 10% error in the nonvolatile constitutents reported on a moisture-free basis. Sampling errors are not specific to thermal methods of moisture analysis, but are inherent in all methods; this is a subject broad enough for consideration in other symposia.

A wide variety of ovens or drying chambers has been designed. To answer the question, "Which ovens are best for moisture analyses?" the following must be considered: Is the moisture present only as surface moisture, is time of drying a factor, and is the moisture deep-seated, as in cells, vacuoles, or capillaries?

If the moisture is loosely held liquid water, almost any type of drying will give satisfactory results. If the water is surface water and time of drying is an important item, elevated drying temperatures are required, but these must be held below the thermal decomposition point of the substance being dried. In drying at atmospheric pressure, time can be gained by using mechanical convection, which removes vaporized moisture from the area of the sample immediately upon vaporization, and reduces to a minimum the time for diffusion of the vaporized water. Drying is usually about four times as fast in mechanical convection ovens as in gravity convection ovens. The former also tend to maintain much greater uniformity of temperature throughout the oven drying chamber. When the moisture is deep-seated

and must diffuse largely through the capillaries, a decided advantage may be gained through the use of vacuum drying.

CONCLUSIONS

Ideal conditions for thermal drying are: using diminished pressure to minimize time of diffusion of deep-seated moisture; using properly desiccated air to sweep the water vapor from the drying chamber; and heating the specimen at a temperature as close as possible to that at which the rate of thermal decomposition becomes appreciable. These conditions provide the maximum difference between the vapor pressure of the water in or on the substance and that of the air in the dryer. It is the magnitude of this difference, together with changes in moisture-vapor diffusion rates, that determines the rate of drying and the degree of drying possible by thermal methods.

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Determination of Moisture by Distillation

W. R. FETZER, *Clinton Foods Inc., Clinton, Iowa*

The determination of moisture, independent of other volatile materials, has always been a problem to the analytical chemist, particularly when it involves a commercial transaction or the yield or recovery in a factory process. The distillation procedure for moisture determination measures the water as such and thereby establishes a primary or reference method for other moisture methods. The average analyst is not familiar with the versatility of the method with respect to apparatus design and too often does not use the best design for his test. This article reviews the numerous applications of the distillation procedure, shows some of the basic designs in apparatus which will adapt the procedure to a specific product, and summarizes the best technique developed for its performance.

THE analytical determination which is used more than any other is the determination of moisture. The moisture content is usually given in all analysis reports and, although it may be a part of the general analysis which shows the quality, purity, or grade of the material, it takes on added significance, for it is the direct measure of the dry substance involved in commercial transactions.

Moisture is most commonly determined as the loss in weight of a material dried in an air oven at a temperature slightly above the boiling point of water or in a vacuum oven at a lower temperature for heat-sensitive materials. In spite of many objections which can be raised against it, oven drying continues to be extensively used, probably for two reasons:

Every laboratory has one or more ovens and the technique is simple, requiring no special training to operate.
Laboratory directors are reluctant to accept a new procedure until it has been thoroughly "seasoned" or to spend funds for the purchase of additional equipment.

Although oven drying methods are satisfactory for many materials, organic or inorganic, they are totally unsatisfactory for many materials which are heat labile or which contain volatile materials other than water.

Many attempts have been made to determine moisture by physical means, such as conductivity and capacity, in order to reduce laboratory cost. Unfortunately, these methods must be standardized or calibrated against another method or procedure before they can be put to use. To determine true moisture, to ascertain whether the product can be handled in an oven, or to calibrate an oven or other moisture apparatus, the distillation procedures are supreme.

Distillation methods have been used for 50 years for the determination of moisture. The recent surge of interest in their use probably stems from three facts:

Introduction of standard-taper joints, which simplify the assembly of condenser, traps, and boiling flasks and eliminate the loss of water through cork closures.
Interest in the true moisture of materials.
The need for a standard reference or an absolute method for the standardization of ovens or other specialized apparatus.

Principle of Distillation Procedure. The sample under test is distilled with an excess of a liquid which usually has a boiling point higher than water and is immiscible with it. Heat is applied to the mass. Water and liquid are distilled off, the excess liquid as vapor removing the last traces of water and driving it from the boiling flask. The combined vapors are condensed and collected, and the separated water is measured.

As the boiling liquid carries the heat effectively and rapidly to all particles of the sample, the water is removed quickly. Because the test is made in an inert atmosphere, all danger of oxidation of the sample by air is removed. With the advent of a refluxing type of condenser, the amount of liquid required for the test is small. However, any chemical reaction in the material produced by heat is not eliminated, although those of the Maillard type are minimized through the quick removal of the moisture. Further danger from this point can be lessened through the choice of a liquid with a lower boiling point, and even a liquid with a boiling point below that of water, such as benzene, can be used, although such a choice extends the time for the test. A large number of organic liquids have been used: benzene, toluene, xylene, turpentine, mineral oil, petroleum fractions, carbon tetrachloride, tetrachloroethane, perchloroethylene, *o*-dichlorobenzene, etc. The first three are the most popular.

The distillation apparatus in its simplest form consists of a boiling flask for the sample and boiling liquid, a condenser for condensing both the water and the liquid, and a trap which collects and measures the liberated water. The trap is so constructed that the condensed liquid flows back into the boiling flask; the condenser functions as a refluxing unit, which enables the last traces of moisture to be removed from the sample and the excess of distilled liquid to wash these traces of water into the measuring trap.

When the boiling liquid is lighter than water, the trap usually contains a calibrated tube, sealed at the bottom and calibrated upward from the end, with a small liquid reservoir above the calibrated portion. This form requires only one meniscus to be read in obtaining the collected water. The calibrated portion of the tube may be cooled by a water bath to the standardization temperature of the calibration, or, as the water is "sealed" by the liquid, the entire trap may be removed for this operation. Objections have been raised against flammable liquids, and nonflammable liquids such as carbon tetrachloride have been proposed. Because these liquids are heavier than water, their use necessitates a different type of trap, for the collected water remains on the liquid, and the liquid must be withdrawn from beneath the collected water and returned to the boiling flask. All the condensed liquid must pass through the collected water, and this is of some disadvantage from the standpoint of temperature in the measurement of the collected water. The possibility of emulsion formation, the lack of a "seal" on the surface of the collected water, and the necessity for reading two menisci for determining the amount of collected water are other disadvantages.

The distillation method for moisture determination has its proponents and opponents. The critics have one point in common —that it is not adaptable as a routine procedure. Basically this criticism can be condensed to the simple fact that distillation requires more space and more man-hours than oven procedures. If the product can be handled by an oven procedure, this method should be employed in the interest of economy.

Complaints about the distillation procedure can generally be answered in two ways:

There had been failure to take into consideration some special technique required for the materials under test.
The fact had been overlooked that some materials are so heat-sensitive that liquids such as benzene or mixtures of benzene and toluene of lower boiling point are necessary.

HISTORICAL

The literature on the distillation procedure is extensive. In most cases, the technique has been applied to materials which have caused trouble with the usual oven procedures, and the investigator has resorted to the distillation procedure to obtain true moisture.

Hoffman (*39*) in 1902 described the distillation method, which was based on a German patent issued in the previous year. It was originally designed for the determination of moisture in cereals and cereal products and used petroleum fractions and toluene as its distilling liquid. Thorner (*72*) applied it to other foods, Gray (*36*) to other products, Schwalbe (*65*) to cellulose products, and Gräfe (*35*) to lignite. Hoffman (*39*) in 1908 presented a resume on the distillation procedure up to that time. He included data on hops, showing that the distillation method with turpentine and toluene gave results which agreed very closely with those obtained by the older methods of drying in vacuo with phosphorus pentoxide or in drying chambers at 80° C. He stated that for any substance in which water is to be determined, at least one of the ten variations of the distillation procedure mentioned in his article was suitable.

In this country, Brown and Duvel (*15*) applied the distillation method to the determination of moisture in grain. They wished to shorten the official procedure, which called for loss in weight of the whole grain when dried in a water-jacketed oven (99–100° C.) for 96 to 120 hours. Their method called for a mineral oil as the distilling liquid, and a time and temperature set to yield the same results as the water oven. Their method, which reduced the test time to 30 minutes, was immediately successful and is today the official method for the purchase of grain. The Tag-Heppenstall moisture meter, which is in wide use, is standardized against the Brown-Duvel method.

Marcusson (*52*) worked with oils, fats, soaps, etc. (in 1905), and used xylene or benzene as his immiscible liquids. Testoni (*71*) made determinations on molasses by distillation in 1904.

Schwalbe (*65*) in 1908 reported the application of the distillation method to plant materials. "In examination of dense materials, it is important to select for the liquid of high boiling point one such that the tension of the aqueous vapor above 100° may be great enough to enable the bubbles of steam to overcome the resistance of passing through the mass." Many workers appear to have taken this view, but it is not necessarily true with adequate dispersion.

In 1910 Sadtler (*62*) reported on the use of benzene for distillation of water from cheese, egg albumen, etc. Folpmers (*32*) used benzene, toluene, and xylene in his work on spices, reported in 1916. Sindall (*68*) tried kerosene on spices previous to 1917 and Besson (*10*) used "petroleum spirit" on cheese and soap about the same time.

In 1917, van der Linden, Kauffman, and Leistra (*47*) published their results of distillation of molasses and other sugar factory products. They observed that xylene gave very satisfactory results, provided a certain rate of distillation was observed, but that prolonged heating caused decomposition of the product with formation of water. They further stated that other volatile immiscible liquids, such as benzene, toluene, kerosene, and mixtures of these gave less satisfactory results.

Earlier workers apparently failed to make the liquids of lower boiling point work satisfactorily on viscous materials because of apparatus limitations and no sample dispersion. The Dean and Stark (*22*) "distilling receiver tube" had not been developed, nor had the improvements in dispersion due to Rice (*60*) and Fetzer, Evans, and Longenecker (*30*) made their appearance.

The development of the Dean and Stark device, which permits continuous refluxing and separation of the water, was a great step forward, as it provided means of removing the last traces of water efficiently. In 1925, Bidwell and Sterling (*11*) improved the design for precise work and their well-known "traps" are now standard equipment.

Norman (*56*) presented work on fats, oils, soaps, etc., in 1925 and drew attention to a special still head provided with a built-in reflux condenser of simple design to permit continuous refluxing. Accurate results were claimed and the use of benzene instead of xylene was recommended.

Yamada and Koshitaka (*77*) modified Norman's apparatus and recommended it for water determination of crude camphor in 1927. They claimed that adhesion of condensed water on the wall of the still head can be completely avoided by a special still head with built-in reflux condenser having an inverted part at its end. The use of toluene or xylene instead of benzene was recommended for camphor.

In 1926, Dedlow and Smith (*23*) found that xylene distillation caused decomposition when applied to meat extract and successfully modified the method by conducting the distillation with xylene under vacuum. This is an interesting variation and gives a flexibility which should prove useful for many products, although it is a little complicated for routine work.

In 1929, Rice (*60*) contributed much to the successful operation of the distillation method for sugar products by placing Filter Cel in the distillation flask and running the sirup in on top. This very useful technique was further developed in 1935 by Fetzer, Evans, and Longenecker (*30*) to apply to extremely viscous materials, such as high-gravity corn sirup. Trusler (*73*) in 1940 presented a very interesting study which reported on the use of benzene, toluene, and xylene with soaps.

In a series of five papers Evans, Cleland, and Fetzer (*20*, *27*) reported a comprehensive research on the moisture determination in sugar products: cornstarch sirup, cornstarch sugar, hydrol (corn sugar molasses), and blackstrap molasses. These products cover a wide range of products which cause trouble in the usual oven procedure. Corn sirup, unless adequately dispersed on a medium such as Filter Cel, surface-dries to a hard

mass, leaving internal moisture which can be released only with difficulty. Hydrol is a heat-labile material containing dextrose, reversion products of dextrose, about 10% ash, about 0.4% protein (dry basis), and considerable acidic residues. They found that toluene could be used as the distilling liquid for corn sirup but that benzene was best for hydrol and blackstrap molasses.

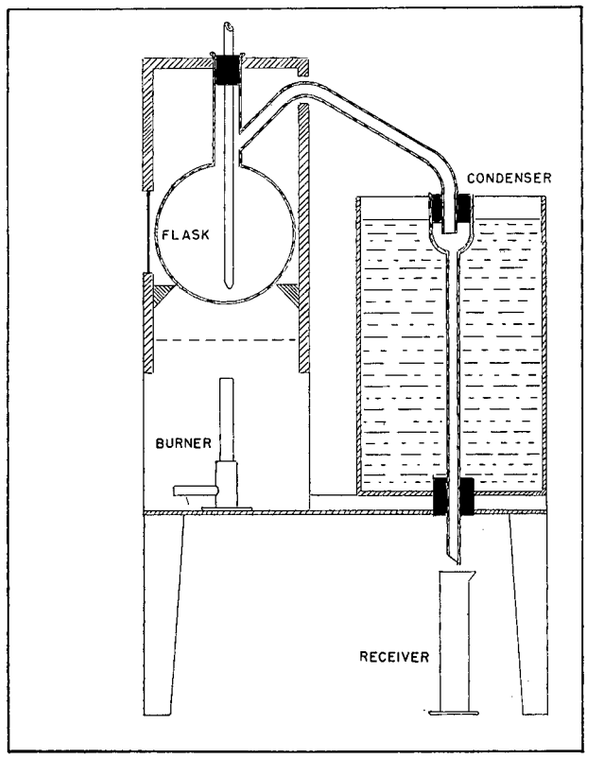

Figure 1. Brown and Duvel Apparatus

Sair and Fetzer (*63*) investigated the moisture determination for corn and the wet milling by-products: corn gluten feed, corn gluten meal, and steep water. Their work on corn showed that the distillation method employing toluene gave true moisture, provided the corn kernel was cracked to about 10 mesh. They presented data for predrying corn containing more than 20% moisture. Corn is often received with 25 to 30% moisture in the fall of the year; occasional carlots exceed these figures. These data showed that the Brown-Duvel method gave low moisture results varying from 1.5 to 2%, depending upon the moisture content of the corn. The Sair-Fetzer method of distillation is the official method for moisture for internal accounting within the wet milling industry, and vacuum ovens at 70° C. are standardized for time and temperature against it.

In 1936, Alexander (*1*) reported on an interesting variation in which a volatile liquid heavier than water—i.e., carbon tetrachloride—was used with a special tube adapted to the reverse of usual conditions. The paper stated that the method has long been in use in laboratories of the Hercules Powder Co. for routine moisture determinations on dynamite. This liquid has some advantages, notably nonflammability.

Among other contributions to this subject, arranged chronologically, are:

Marcusson (*52*) lubricating grease; Rogers (*61*) leather, employing toluene; Michel (*53*) foods; Sindall (*68*) spices; Fuchs (*33*) benzene for mineral oils; Blythe (*12*) sulfonated oils; Barber (*7*) sulfonated oils by 100° C. oven, with paraffin, benzene, toluene, gasoline, and xylene; Schwalbe (*65*) further work on cellulose products; Feder (*28*) sausage; Myhill (*55*) coals with benzene, toluene, and xylene; Sindall (*68*) further work on spices; Bakker and Stunhauer (*6*) foods and spices; Drefahl (*26*) wood preservatives; Dohmer (*25*) case-hardening materials; Holtappel (*41*) foods with xylene; Jones and McLachlan (*44*) comparison data on toluene distillation; Boller (*13*) hydrocarbons; do Couto (*21*) oils and tar; Calderwood and Piechowski (*18*) distillation technique; DeLoureiro (*24*) improvements in technique; Wefelscheid (*75*) xylene for greases; Lownes (*50*) animal and plant tissue employing xylene; Holt and Callow (*40, 50*) tumor tissue; Woodmansee, Rapp, and McHargue (*76*) a comparison of three methods on tobacco—oven at 65°, 100°, and 135° C., room temperature over sulfuric acid, and toluene distillation; Miller (*54*) water in blood by toluene distillation; Locket and Barrett (*49*) water in soils employing toluene and xylene; Gough and Green (*34*) sand bitumen and sand tar carpets; Bailey (*5*) self-rising flour and pancake, waffle, and doughnut flours; Brown (*16*) crude phosphorus and sludges containing phosphorus.

Horner (*42*) patented a distillation apparatus for determining the moisture of drilling core samples. Bruening (*17*) reported on cosmetic creams, employing toluene; Maercklein (*51*) deodorants and antiperspirants. Hallsworth (*38*) found that on plant materials toluene distillation gave higher results than vacuum oven (70° C.) but less than air oven. Johnston used chloroform as the boiling liquid in determining the moisture in dehydrated foods. Simon (*67*) describes an apparatus for determining the moisture in coal, which resembles the ASTM apparatus for determining water in mineral oil. Simek and Ludmila (*66*) describe a special apparatus for coal; benzene, toluene, and xylene gave identical results. Churchward (*19*) gives results on foods and prefers petroleum fractions (boiling point 105° to 110° C.) as the boiling liquid. Berthelot (*9*) describes special considerations for determining the water in peat. Fisher and Hauser (*31*) describe a special apparatus and method for drugs. Miller (*54*) gives a modification for animal tissue. Perkins (*57*) employs oven and distillation procedures on silage, with the conclusion that the difference is a measure for volatile materials other than water. Kauffman and Keller (*45*) determine the moisture in oil seeds employing heptane (boiling point 98° C.), reserving the heptane for subsequent fat determination. Brodshil (*14*) uses bromobenzene, toluene, and xylene as distilling liquids on foods. Phillips and Enas (*58*) describe a special apparatus with tetrachloroethane as the boiling liquid.

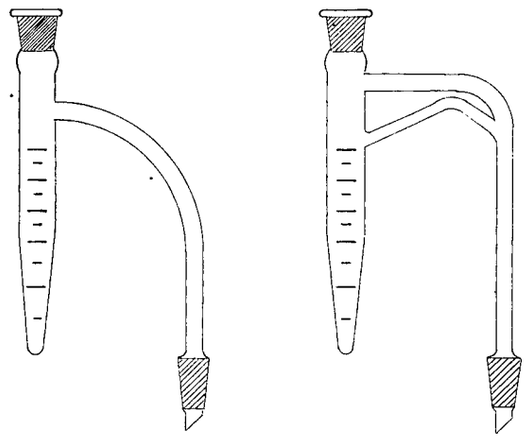

Figure 2. Dean and Stark (*Left*) and Modified Dean and Stark Trap (*Right*)

Baumgarten, Stone, and Boruff (*8*) in a comparison of the distillation procedure against other methods found that the pH of the water collected when employing toluene and xylene was higher than when chloroform or benzene was used. The distillate gave a test for nitrogenous material by Nessler's reagent. Picozzi (*59*) used the distillation procedure for water in soaps and fatty acids. Schley (*64*) recommends the use of a small amount of wetting agent in the trap to obtain a better meniscus. Hruda (*43*) employed a copper boiling flask with xylene for coal, raw sugar, beet seed, and cossettes. Steagall (*69*) studied the moisture in flour, employing benzene distillation with results comparable to vacuum oven at 70° C. Lindsay (*48*) describes a special trap for relatively dry materials. Weeks (*74*) reports results on the distillation procedure for cosmetics, but does not recommend the method because of its cumbersomeness and necessity for cleanliness. Fedorov (*29*) describes a special distillation apparatus. Hadorn (*37*) reports results on fruit juices

employing perchloroethylene and toluene, but reports caramelization of the sugar. Talon (*70*) gives a comparison of air oven, vacuum oven, and room temperature over sulfuric acid, with distillation with xylene.

APPARATUS

Many of the published papers deal with apparatus, particularly the design of moisture receivers or traps. Some of the suggested designs have been so widely accepted that they are stock items in the usual laboratory supply catalog and bear the inventor's name. Others, although successful, were designed for a specific purpose and must be built from designs found in the original paper. The standard apparatus and some of the suggested modifications are described here.

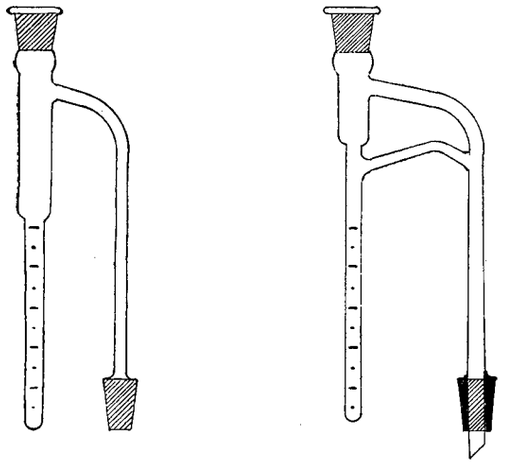

Figure 3. Bidwell and Sterling (*Left*) and Modified Bidwell and Sterling Trap (*Right*)

For Distilling Liquids Lighter Than Water. BROWN-DUVEL. This apparatus has changed very little since it was introduced 42 years ago. The boiling flask is of copper and there is no provision for refluxing the distilling liquid. The apparatus is simple and gives surprising precision between operators (Figure 1).

DEAN-STARK. The Dean-Stark trap (1920) changed the distillation procedure from a simple distillation to a multiple distillation by a change in design whereby a refluxing condenser was introduced (Figure 2). This change resulted in a smaller boiling flask and required less distilling liquid. It renewed the interest in the distillation procedure, for it enabled the last traces of water, which have always been the moot point in any moisture controversy, to be removed and measured.

The modification of the Dean-Stark trap shown on the right of Figure 2 was introduced to channel the droplets of water more effectively into the calibrated portion of the trap. Although it was effective, it greatly increased the difficulty in cleaning the traps and most users prefer the original trap.

BIDWELL-STERLING. In 1925 Bidwell and Sterling improved the Dean and Stark tube to overcome certain difficulties, and these changes were so successful that it is probably the trap in widest use (Figure 3). For this reason it has been classified as a trap type rather than a Dean and Stark modification. This trap has a small reservoir above the calibrated tube, which slows down the return of the distilled liquid and enables the droplets of water to fall into the measuring tube. The measuring tube resembles a Mohr pipet and is usually made in two sizes—5 and 10 ml.

To the right in Figure 3 is shown a common variation. The same comments can be applied here as above.

CLELAND AND FETZER. Materials which contain a small amount of moisture can usually be determined with more precision, as a larger sample can be used. This is also true with the distillation procedure. The precision of any moisture determination falls as the amount of water increases, and this has posed a problem for the distillation procedure where traps and boiling flasks must be kept within certain space limits. Evans and Fetzer (*27*), in a research on dilute sugar sirups, modified the Bidwell-Sterling trap by an ovoid bulb sealed to the usual calibrated 5-ml. tube, giving a total capacity of 20, 25, and 30 ml. (Figure 4). The ovoid bulb was chosen to prevent occlusion of the distilling liquid at the junction of the bulb and calibrated stem. These tubes worked satisfactorily, but presented difficulties in cleaning.

For Distilling Liquids Heavier Than Water. Traps for such liquids present some difficulty, as the distilling liquid must pass through the collected water in its return to the boiling flask. This requires that the return tube be placed in relationship to the density of the distilling liquid. This construction limitation is discussed by Langeland and Pratt (*46*).

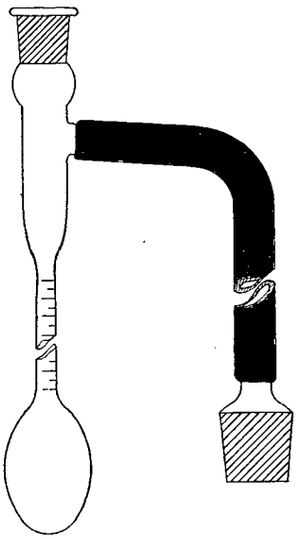

Figure 4. Cleland and Fetzer Trap

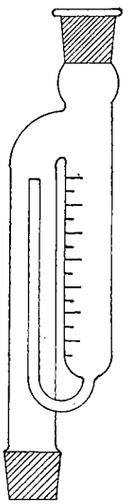

Figure 5. Hercules Trap

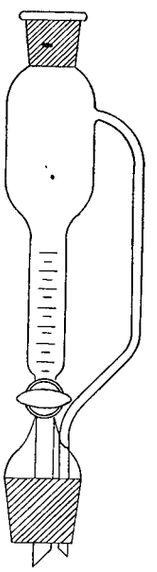

Figure 6. Bailey Trap

HERCULES TRAP. Alexander (*1*) introduced this trap in 1936. It has wide acceptance and is a standard item in supply catalogs. Its construction features are clearly shown (Figure 5).

BAILEY TRAP. Bailey (*4*), in 1937, published the design of a trap for determining moisture in wood chips (Figure 6). The distilling liquid was tetrachloroethylene and the design required that the distilling liquid be returned periodically to the boiling flask through a stopcock below the calibrated tube.

LANGELAND-PRATT TRAP. Langeland and Pratt in 1938 published their design for a trap for distilling liquids heavier than water (Figure 7). The research was based on a desire to avoid the manual return of distilling liquid required by the Bailey trap, and to design a continuous return of distilling liquid. This trap is similar to the ASTM trap (*2*) used for oil dilution and the Clevenger trap for the determination of oils lighter than water.

ANGLE TRAP. The inventor of this trap is not known, but it has become a standard type. The design puts the calibrated tube on an angle, which permits the distilled liquid to pass the collected water along the tube surface rather than through the body of the collected water. This change speeds the return of the distilling liquid to the boiling flask and produces less turbulence in the collected water.

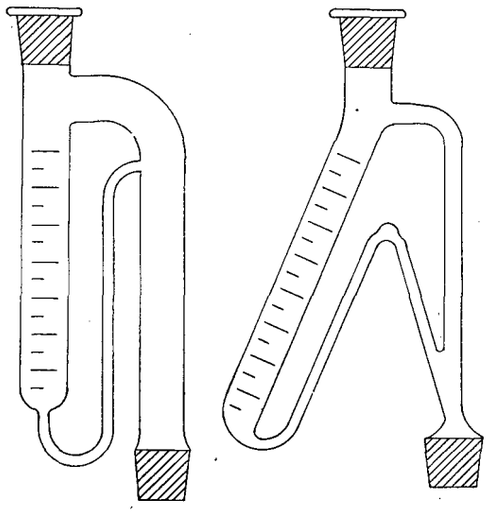

Figure 7. Langeland Pratt (*Left*) and Angle Trap (*Right*)

Typical examples of apparatus designed to solve special problems are shown in Figures 8 to 16.

DISTILLATION TECHNIQUES

The success of the distillation method depends primarily on the selection of the best equipment, and once these are obtained, upon certain tricks in sample handling and procedure. The resume of these various points is based on the author's personal experience and interchange of ideas on this subject with others.

Apparatus. BOILING FLASK, 250-ml. Erlenmeyer flask, Pyrex glass No. 7740 45/50 ⅄ joint. A flask with these specifications has several advantages. It is of sufficient size for most samples; the wide mouth permits the use of a pestle for the incorporation of viscous samples with the dispersing agent; it is easily cleaned; and it can be weighed on an analytical balance when the sample must be introduced into the flask and the net weight obtained by difference.

TRAP, Bidwell-Sterling 5-ml. Pyrex ⅄ 24/40 and 45/50 joints (Figure 17). The vapor tube between the ⅄ 45/50 joint and the trap reservoir should have an inside diameter of 10 mm. and be lagged with several layers of electrician's tape and subsequently lacquered or shellacked. The insulation, although small, greatly reduces the condensation of the distilling liquid in the vapor tube, resulting in a lower bath temperature to produce the required amount of refluxing in the condenser. A lower bath temperature decreases the tendency of "bumping" with possible carry-over of sample or dispersing agent.

CONDENSER, West type, ⅄ 24/40 joint, 30-cm. jacket, water-cooled joint, and a drip tip. The West-type joint is recommended for lightness and ease of assembly. The water-cooled joint lessens the evaporation of distilling liquid at the joint. The tapered drip tip is a "must" and should be designed so that droplets of water, on leaving the tip, fall into the center of the reservoir. This centering eliminates to a very large extent the collection of droplets of water on the sides of the reservoir.

CONDENSER CAP, a standard buret cap or a small test tube. The condenser should be capped during the distillation, particularly in the summer months, to prevent the condensation of atmospheric moisture.

CAPS FOR CLEANING, ⅄ 45/50 cap and ⅄ 24/40 Pyrex. These caps are an aid in the cleaning of the trap and condenser with cleaning acid (dichromate-sulfuric acid), as they enable the traps and condenser to be filled with the agent and allowed to stand for the necessary time to effect cleanliness (Figure 18).

BATH, the preferred method of heating the flask is by an oil or wax bath. Such a bath carries the heat uniformly to the flask. The level of the bath or wax should be above the level of the distilling liquid in the boiling flask, for this simple precaution reduces bumping.

These specifications appear to be fairly simple. However, the Technical Advisory Committee of the Corn Industries Research Foundation, which has investigated analytical procedure in the wet milling industry for over 15 years, found that it was necessary to go even further in order to secure uniformity between laboratories. They have cooperated with an apparatus maker who now furnishes the complete distillation assembly according

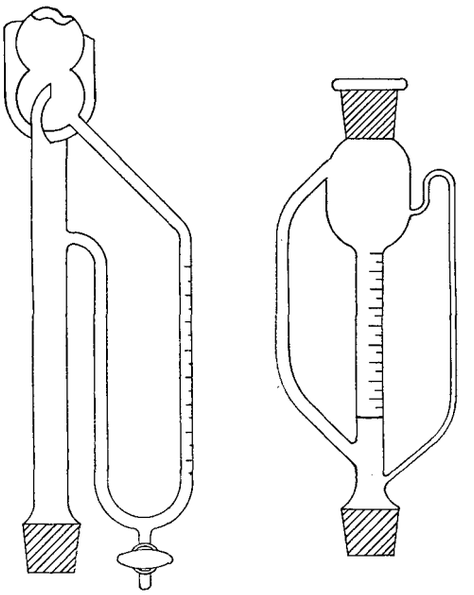

Figure 8. Solvents in Paint
Commercial model on right

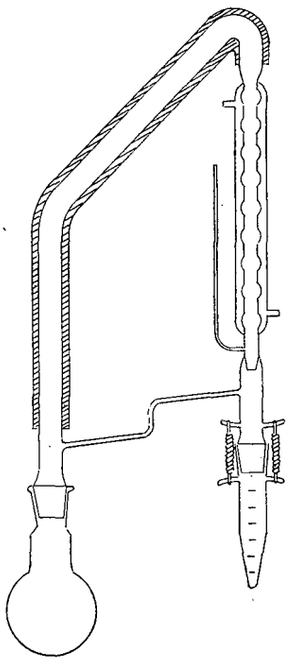

Figure 9. Marskell and Rayner Apparatus

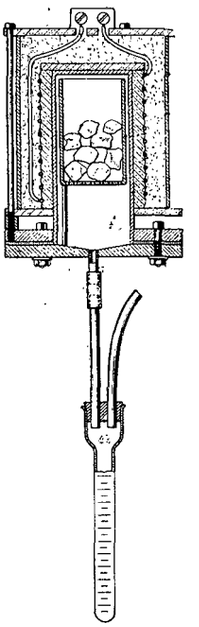

Figure 10. Horner Apparatus for Fluids in Solids

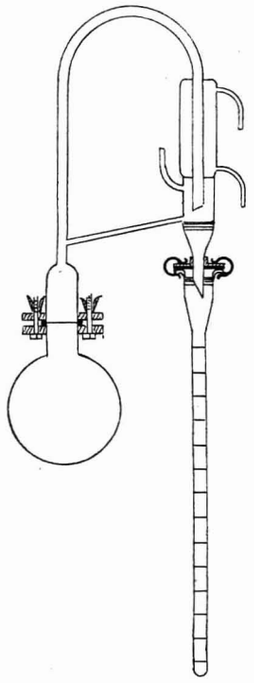

Figure 11. Simek and Ludmila Apparatus

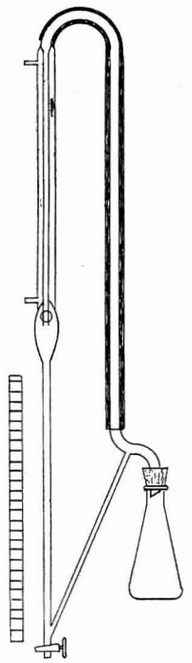

Figure 12. Phillips and Enas Apparatus

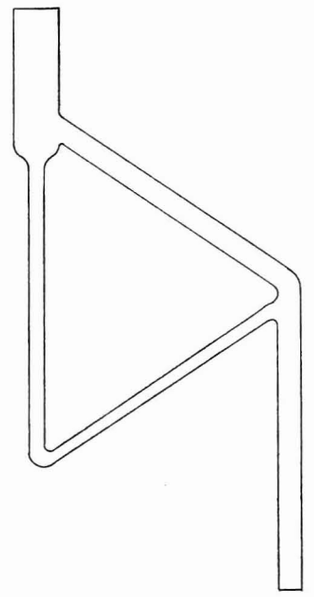

Figure 13. Johnston Apparatus

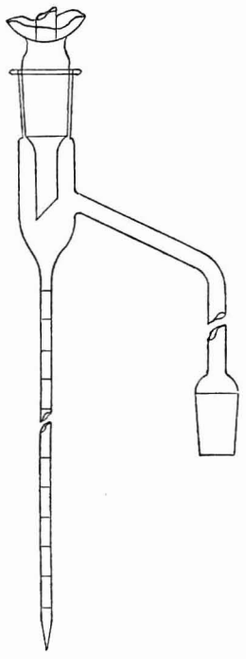

Figure 14. Miller Apparatus

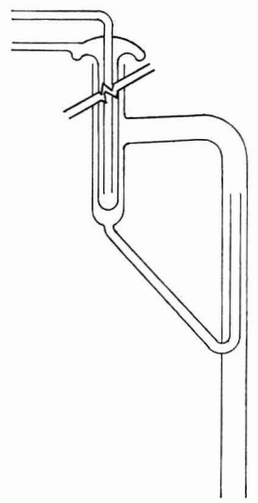

Figure 15. Lindsay Apparatus

to detailed specifications. This assembly contains six units and resembles an oil-extraction rack (Figure 19).

The distillation method will give high precision in the determination of moisture if reasonable care is taken to assure clean apparatus and the user takes advantage of several simple precautions.

Cleaning of Apparatus. The common complaint against the distillation method is the necessity for clean apparatus. If the trap or condenser is greasy, a small amount of water may be held on the inner surface of the condenser or on the sides of the trap reservoir.

Traps and condenser may be easily cleaned by means of any of the kitchen detergents and a long-stemmed brush. They may all be thoroughly cleaned by applying the standard-taper caps, filling with cleaning solution, and allowing to stand. In order to do this, it is necessary to carry several extra traps and condensers. After either of the above treatments, the apparatus should be washed with distilled water and placed in an air oven to dry. If it is necessary to store clean apparatus, it should be plugged with cotton wool.

Treatment of Standard-Taper Joints. Before assembling the apparatus, each $\overline{\$}$ joint should be marked with a No. 2 pencil. Closure should be made, with a few drops of the distilling liquid, and a firm seal should be made by turning the joints. In this way, the pencil graphite is spread between the joint surfaces, enabling them to be easily separated on the completion of the test.

Dispersing Agents. It has long been the practice in oven procedures to disperse on sand materials, which surface dry and harden, in order to assure complete moisture removal. This is also necessary in the distillation procedure. For this purpose, diatomaceous earth or Filter-Cel is an ideal medium and the wide-mouthed boiling flasks are most suitable.

The flask and Filter Cel are dried and weighed. The sample is placed on the Filter Cel and the flask is reweighed. It is then worked into the Filter Cel, employing a small test tube or vial as a pestle; a pencil inserted in it serves as the handle. After the initial mixing, the mass should be lubricated with a small amount of distilling liquid. This step is important, for the quickness and completeness of water removal are dependent upon thoroughness of this procedure for many difficult materials such as sugar sirups and products containing protein. The vial or test tube is left in the boiling flask.

Many materials in a powder form, such as cereals, flours, and starches, have a tendency to bump during the distillation, through superheating on the bottom of the flask. This can be largely overcome through the introduction of a small amount of dry short-fiber asbestos.

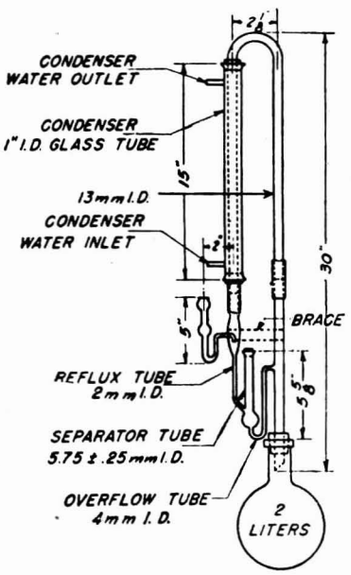

Figure 16. Chemical Corp. Apparatus

Distilling Rate. The distilling rate of 3 drops per second, employed by Bidwell and Sterling, has been found to be best. Higher rates, particularly at the start of the distillation, drive the water vapor too high in the condenser for subsequent collection by the distilling liquid.

Wetting Agents. In distilling some materials such as cereal grains, a small

amount of fat or wax is often carried into the reservoir trap or the drip tip of the condenser. This condition sometimes causes an isolated droplet of water to cling to these surfaces. These can be removed mechanically through a squeegee, made from a narrow band of 1/8 inch rubber tubing, which has been cut and attached to a small wire. This device also will correct a faulty meniscus. Langeland and Pratt (*46*) and Schley (*64*) suggest the use of a small amount of wetting agent to secure a better meniscus reading.

Distilling Liquid. The choice of the distilling liquid or boiling temperature becomes important. The author believes that toluene (boiling point 110° to 112° C.) is high enough for most materials if the sample is dispersed. In the literature, where distilling liquids with a higher boiling point than toluene have been employed, the investigators were probably forced to this necessity, because they failed to utilize the effective technique of sample dispersion. If the material is heat-sensitive, benzene or mixtures of benzene and toluene should be used.

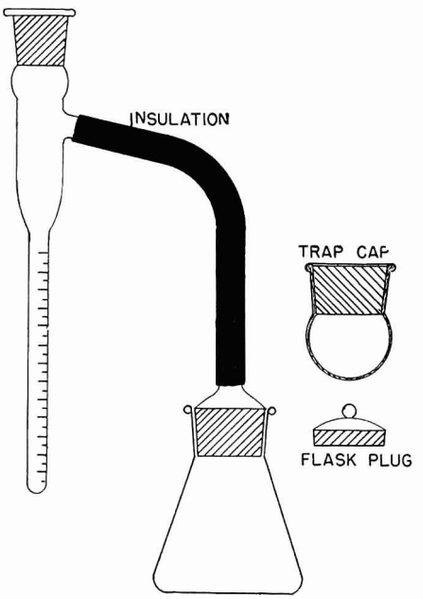

Figure 17. Cleland and Fetzer Apparatus

A blank must be run on the distilling liquid, and although this is usually constant for a good grade or make, it is suggested that a fairly good sized quantity be obtained so that blank determinations need not be a routine matter.

The sample size should be chosen, if possible, so that the amount of collected water falls within a 3.5- to 5.0-ml. range, thereby assuring greater precision.

SUMMARY

The distillation procedure for moisture determination, has been used on materials which could not be analyzed or gave questionable results in the usual oven procedures. With this in mind, there are several points to remember.

The distillation procedure for moisture should not be used if the conventional oven methods are applicable.

If the distillation procedure is necessary, equipment should be carefully chosen and every effort made to follow the suggestions that have been found advantageous in the distillation technique.

If the material is heat-sensitive, it is often possible to standardize an oven with respect to time and temperature, so that the oven method will give close agreement with the distillation method.

The distillation procedure for moisture is official for many materials in ASTM (*2*) and AOAC (*3*) procedures.

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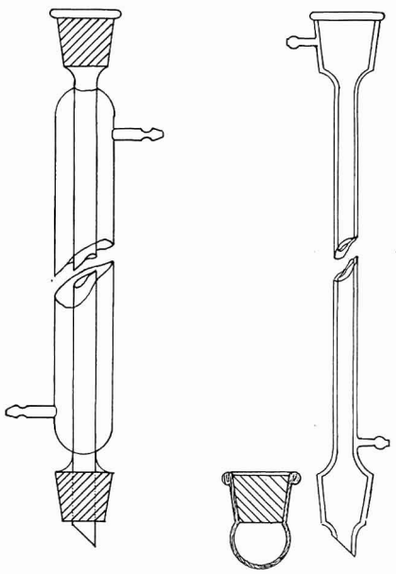

Figure 18. Condensers

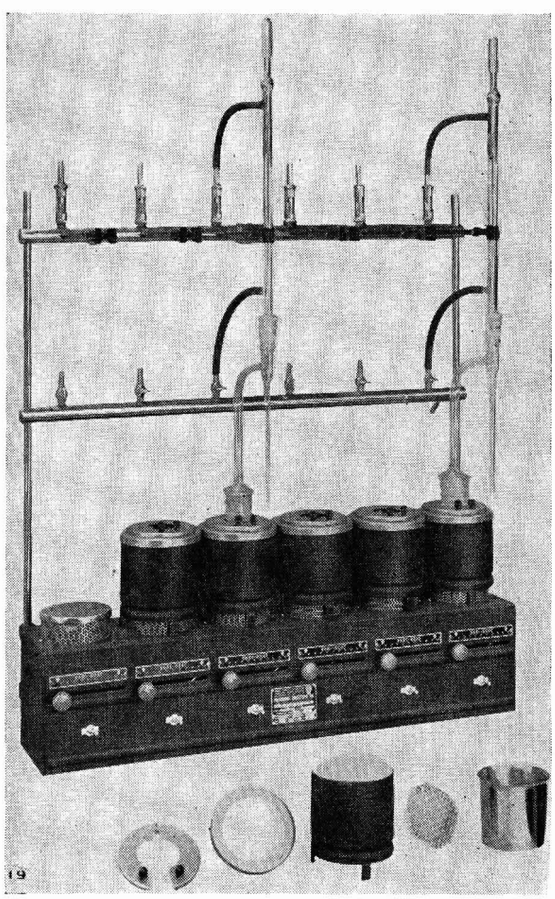

Figure 19. C.I.R.F. Distillation Rack

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Karl Fischer Reagent Titration

JOHN MITCHELL, JR.

Polychemicals Department, Research Division, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

BOTH physical and chemical procedures are available for determining water. The choice of the technique best suited for a particular problem is dependent on several factors, including sensitivity and the precision and accuracy required, facilities available, and the nature of the materials to be analyzed. Thus in the lumber industry where facilities often are limited and high accuracy is not essential, many types of direct-reading instruments are available which depend on some electrical property, such as dielectric constant, conductance, or capacitance. Most physical methods are based on direct removal of water, after which the anhydrous residue is weighed and moisture is estimated by difference, or the water is recovered and measured volumetrically or gravimetrically. The former condition is most often represented in oven drying. The latter condition is the basis of most azeotropic distillation techniques, where the recovered water is measured, or of volatilization methods, where moisture is absorbed on an active desiccant. Obviously all these methods fail on materials that are thermally unstable.

Numerous chemical methods have been proposed for the determination of water. Actually, those physical separations employing recovery of moisture on a desiccant are partly chemical in nature, as this absorption usually requires chemical reaction either by hydrate formation or by hydrolysis. The most widely applicable chemical method is that based on the Karl Fischer reagent. Indeed, it can be stated unequivocally that the Karl Fischer technique may be employed successfully for the determination of moisture in many more types of materials than any other existing method. The nearly specific nature of this reagent, together with the rapidity with which analyses can be performed, is making the Fischer reagent an essential tool in the analytical laboratory. However, it can be used effectively only when its great potential and limitations are clearly understood.

Karl Fischer's initial investigations were based primarily on the need for a reliable method for moisture in petroleum chemicals and sulfur dioxide. Since its publication in 1935, the Fischer method has been the subject of an increasing number of publications describing new fields in which this technique has found successful application.

Fischer reagent is composed of iodine, sulfur dioxide, pyridine, and methanol. Contrary to some reports, each of these compounds enters into the basic reaction with water. The over-all process involves the two-step reaction (*32*):

$$C_5H_5N.I_2 + C_5H_5N.SO_2 + C_5H_5N + H_2O \longrightarrow 2C_5H_5N.HI + C_5H_5N.SO_3 \quad (1)$$

and

$$C_5H_5N.SO_3 + CH_3OH \longrightarrow C_5H_5N(H)SO_4CH_3 \quad (2)$$

It is apparent that only Equation 1 involves water, while Equation 2 completes reaction of the intermediate, the pyridine–sulfur trioxide complex. Other compounds, containing active hydrogen, can react with this complex. For example, water might be involved, as shown in Equation 3

$$C_5H_5N.SO_3 + H_2O \longrightarrow C_5H_5N(H)SO_4H \quad (3)$$

However, Reaction 3 would be of no practical value for the determination of water. It is not specific for water and is not accompanied by a color change.

The stoichiometric requirements for the components of the Fischer reagent are established as given in Equations 1 and 2—that is, for each mole of water are required 1 mole of iodine, 1 mole of sulfur dioxide, 3 moles of pyridine, and 1 mole of methanol. As it is common practice to employ excess sulfur dioxide, pyridine, and methanol, the strength of any given preparation of reagent is limited by the iodine concentration. The excesses of other components may be varied to meet particular analytical requirements. The author has found that a methanolic solution containing other components in the ratio $1I_2:3SO_2:10C_5H_5N$ at a concentration equivalent to about 3.5 mg. of water per ml. of reagent to be the preferred composition for general work. Other compositions may be better suited for specific purposes—for example, a reagent containing considerably more pyridine may be used for titrations for water in acetone. A reagent containing as little as 4 to 5 moles of pyridine per mole of iodine would be suitable in control titrations for water in alcohol and would effect a significant saving in cost of the reagent.

Numerous variations have been proposed for the preparation of Fischer reagent. In the author's laboratory where large quantities are consumed in all types of applications, the following procedure has been found most efficient (*16*). Stable "stock" solutions are prepared of iodine dissolved in pyridine and then diluted with methanol. Liquid sulfur dioxide is added to portions of the stock solution a day or two before use.

This preparative method is employed to minimize losses of active reagent via parasitic side reactions which consume iodine. In the absence of sulfur dioxide, however, the reagent remains stable indefinitely. For this reason many of the laboratory supply houses market the reagent as two solutions: a solution of iodine in methanol, and sulfur dioxide in pyridine. They recommend that the solutions be mixed shortly before actual use.

For either of the above cases, the complete reagent usually is delivered into the sample to be analyzed. A unique modification was suggested by Johansson (*7*), who prepared two separate solutions of iodine in methanol, and sulfur dioxide in pyridine and methanol. The latter also was used as solvent for the sample and the former as titrant. The iodine in methanol solution was stable and, when used in direct titrations, offered negligible interferences from degradative side reactions. Seaman, McComas, and Allen (*31*) in further studies of this technique demonstrated that, provided adequate protection against moisture was given, each preparation of iodine-in-methanol solution need be standardized only rarely. This standardization was most conveniently made by means of sodium thiosulfate titration. Titrations of samples for water were subject to a small but constant correction for water in the methanol used to prepare the iodine solution.

Seaman and his coworkers made the important observation that no significant side reactions of the Fischer reagent occurred during direct titration—i.e., under conditions in which the iodine reacted immediately with water. When excess iodine was added, side reactions were likely to occur. However, at temperatures of $-10°$ to $-15°$ C. very little decomposition was evident if no more than a 10-ml. excess of reagent was used and the mixture was allowed to stand no more than 10 minutes before back-titration.

Another variation of the two-reagent titration procedure for microdeterminations of water was proposed recently by Johansson (*6*). In order to improve the sensitivity of the titration, he suggested that the hydrogen iodide formed in the reaction be determined, as shown in Equations 4 and 5

$$HI + 3Br_2 + 3H_2O \longrightarrow HIO_3 + 6HBr \quad (4)$$

$$HIO_3 + 5KI + 5HCl \longrightarrow 3I_2 + 3H_2O + 5KCl \quad (5)$$

Iodine thus formed is determined by titration with sodium thiosulfate. Since in the Fischer reagent reaction 1 mole of water forms 2 moles of hydriodic acid, the final titration actually will involve 6 moles of iodine. Johansson reported an accuracy to within 0.04 mg. on samples containing 0.5 to 4 mg. of water.

In the final chapter of the book "Aquametry" it was suggested that bromine could replace iodine in the Karl Fischer reagent. Johansson (*6*) employed this idea to remove water from the pyridine–sulfur dioxide reagent before addition of the sample. However, as bromine alone did not effect a change at the end point, a few microdrops of iodine were introduced and then bromine was added. At the end point, iodide was oxidized to iodine, which resulted in a visual or electrometric change. "Preneutralization" of the solution, therefore, did not effect any significant formation of iodide ion. Then the sample could be introduced into the anhydrous system and iodine added. A direct electrometric end point was employed.

This extension of technique increases the time requirement and probably does not significantly improve the sensitivity of the analysis. Both sensitivity and accuracy are still dependent upon the exactness with which the iodine solution can be added to be equivalent to the water content of the sample.

TITRATION

The method employed for titration—i.e., visual or electrometric—has been the subject of numerous publications, with the advocates of each stoutly pointing out the superiority of one over the other. The electrometric end points are the more sensitive but also more time-consuming. The visual titration requires only simple apparatus and, contrary to the expostulations of the electrometric titration exponents, presents a relatively sharp end point on solutions which are not deeply colored. Most of the difficulties encountered with the visual end point appear to be associated with incomplete titrations. The color changes are canary yellow to chromate yellow to the brown of unused iodine. Often a permanent color standard is desirable when volumes of 250 ml. or more are titrated. Jones (*9*) suggested an arbitrary standard of 0.003 *N* iodine in water solution. The author has found that an approximately 0.01 *N* iodine in methanol solution more closely compares with the true end point.

At the proper end point, the addition of 0.1 to 0.2 ml. of reagent effects a very marked change in color to dark brown. Clear or lightly colored solutions may be titrated easily with a reproducibility and accuracy of better than ±0.2 ml. of Fischer reagent, equivalent to about 0.5 mg. of water. Practically, this error is insignificant in most analytical work.

For example, let us assume we have a solution of alcohol containing 2.00% water. We titrate duplicate 10-ml. (8-gram) samples and find 159.5 and 160.5 mg. of water. Our found figures would be 1.99^+ and 2.01^-%, respectively. Similarly 50 ml. (40 grams) of a sample containing 0.20% water might give 79.5 and 80.5 mg. of water equivalent to 0.199 and 0.201%. However, on the same basis, 50 ml. of a sample containing 0.001% water would give results as high as 0.002%.

The rapid Fischer reagent titration probably represents the most widely applicable technique for the determination of moisture. Either a visual or an electrometric end point may be employed. The former requires only simple apparatus and is sensitive to less than 0.5 mg. of water. The latter requires a desiccant-protected closed system and is sensitive to about 0.2 mg. of water. The "dead-stop" end point employing a direct titration procedure appears to be the most convenient electrometric technique. Organic substances which interfere in the direct titration for water include carbonyl compounds, mercaptans, diacyl peroxides, thio acids, and hydrazines. Usually methods are available to eliminate these interferences. Inorganic compounds which interfere include metal oxides, hydroxides, carbonates, bicarbonates, chromates, dichromates, borates, and sulfides. Often the interfering reactions are quantitative. Several methods based on prior distillation or extraction of the water have been proposed for the determination of water in these inorganic systems. This titrimetric method is becoming increasingly important for the routine determination of water in commercial materials. Reliable methods have been devised for the determination of water in petroleum products, oils, fats, waxes, explosives, paints, polymeric materials, soap, and many foodstuffs and carbohydrates.

If sample size is limited and high accuracy is required, the electrometric end point would be better suited. However, if large quantities of sample are available, two methods may be used with the visual titration.

1. Considerably larger samples might be employed. For example, as much as 500 grams of liquid butadiene may be titrated directly at 0° C. with a reproducibility of better than ±1 p.p.m. A similar precision was observed in the determination of water in adipic acid. Samples weighing 75 to 100 grams were dissolved in 250 ml. of pretitrated 1 to 1 pyridine-methanol. The resulting Fischer reagent titration served as a direct measure of moisture in the sample. Results are shown in Table I. In a series of six successive determinations, figures were 639 ± 4 p.p.m. Another series of seven determinations made over a period of several days showed a maximum deviation of ±10 p.p.m.
2. Direct extraction methods may apply. Thus, Gester (*4*) found that 250 ml. of ethylene glycol could be used to extract traces of moisture from a gallon of hexane.

Table I. Analytical Data for Water in Adipic Acid

Number	Water Found, Wt. %
1	0.0642
2	0.0635
3	0.0642
4	0.0638
5	0.0642
6	0.0635
	Av. 0.0639 ± 0.0004
1	0.056
2	0.056
3	0.056
4	0.058
5	0.057
6	0.057
7	0.060
	Av. 0.057 ± 0.001

EQUIPMENT

The equipment required for visual titration includes an automatic or bottom-filling buret suitably protected against atmospheric moisture by the use of desiccant-packed tubes. Where possible, 250-ml. volumetric or similar long-necked flasks should be used as titrating vessels. The ends of these flasks can be brought up to contact the bottom of the buret stopcock and thereby minimize exposure to the atmosphere. A completely closed system can be made by attaching a spherical inner grind to the base of the buret stopcock and using a flask with a spherical outer grind (*17*). The spherical grind of the flask is attached to the buret stopcock and held in place by a spring clip. Sufficient play is given to permit manual agitation during the titration. This assembly is particularly useful for analyses at reduced temperatures and for the slower titrations of heterogeneous systems,

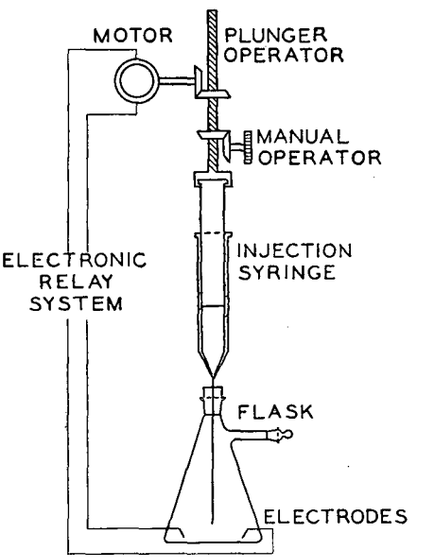


Figure 1. Apparatus for Automatic Titrations

in which reagent is added until an apparent end point is reached and the mixture is allowed to stand to permit further extraction of moisture from the solid phase.

For electrometric titrations the "dead-stop" technique, employing platinum electrodes, is most often used. Instruments based on this principle are sold by many laboratory supply houses. Generally, excess Fischer reagent is added, after which the excess is determined by back-titration with standard water-in-methanol. This procedure was employed originally because the end point appeared sharper. Other investigators—e.g., Carter and Williamson (*2*)—have reported the use of direct methods, employing a greatly increased potential between the platinum electrodes. Probably the use of platinum electrodes of considerably larger diameter ($^1/_8$ to $^3/_{16}$ inch) also would accomplish improved sensitivity. All electrometric methods require a completely closed system (*10*).

The microtitrations are best carried out by electrometric methods. On this scale the novel assembly of Levy, Murtaugh, and Rosenblatt (*11*) is useful. Rubber dental dam is used to seal the tube. The buret is connected to the hypodermic syringe needle which pierces the dental dam. Other needles may be used to introduce sample or act as vent. The platinum electrodes, sealed into the bottom of the tube, are connected by suitable leads to the titrimeter.

Johansson (*6*) described an apparatus for automatic microtitrations. A hypodermic syringe, driven by a worm-gear motor (see Figure 1), was connected to an electronic relay in such a way

Table II. Noninterfering Organic Compounds

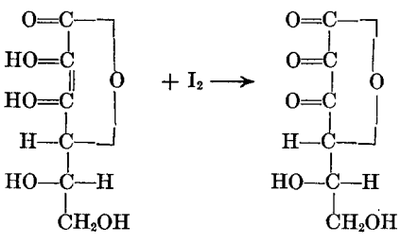
Class	Examples
Acids	Carboxylic, hydroxy-, amino-, sulfonic
Alcohols	Mono-, polyhydric, phenols
Esters	Normal carboxylic, ortho, carbamates, lactones, esters of inorganic acids
Stable carbonyl compounds	Sugars, formaldehyde, benzil, benzoin, chloral
Acetals and ethers	
Hydrocarbons	Saturated, unsaturated (aliphatic and aromatic)
Anhydrides and acyl halides	
Peroxides	Hydro-, dialkyl
Nitrogen compounds	All types
Halides	
Sulfur compounds	Sulfides, thiocyanates, thio esters

that at the end point, as determined electrometrically, the motor instantly stopped. The volume of Fischer reagent employed then could be read from the calibrated screw system. A means for manual operation of the plunger also was provided.

The Karl Fischer reagent may be used for the determination of water in liquids, gases, and solids. However, certain classes of compounds interfere either by reacting with the iodine of the reagent to show apparent water or by oxidizing the hydrogen iodide to form iodine and thus lead to low values for free water.

The types of organic compounds which do not interfere are illustrated in Table II.

Very few exceptions have been observed. One of the more important to the food chemists, at least, is ascorbic acid. Vitamin C is oxidized to dehydroascorbic acid.



$$+ I_2 \longrightarrow \text{(dehydroascorbic acid)} + 2HI \qquad (6)$$

Johnson (*8*) found that the reaction was essentially quantitative. Therefore, the Fischer reagent titer would represent the sum of the moles of water plus ascorbic acid.

In some cases slight modifications in technique are necessary for the titration for water in certain organic materials. Many of these compounds when nearly anhydrous exhibit a false end point. This is effectively eliminated in all cases by use of an inert solvent for both sample and Fischer reagent end products. Methanol or pyridine is suitable. As a matter of fact, all titrations should be made in the presence of an inert solvent in order to blank out the moisture originally present in the titration flask. Volatile compounds often may be titrated at temperatures below their boiling points—for example, methyl chloride at −30° to −40° C. In other cases the moisture may be extracted and the anhydrous sample evaporated, after which the extractant may be titrated at room temperature. Moisture in ammonia gas may be determined in this way.

Most carbonyl compounds interfere. Active aldehydes and ketones tend to react with the methanol of Fischer reagent to form acetals and release water.

$$RCHO + 2CH_3OH \longrightarrow RCH(OCH_3)_2 + H_2O \qquad (7)$$

$$RR'CO + 2CH_3OH \longrightarrow RR'C(OCH_3)_2 + H_2O \qquad (8)$$

Their interference is evidenced by a fading end point. In many cases the rate of the interfering reaction may be reduced sufficiently by use of pyridine as solvent to permit a reasonably reliable rapid titration for water. In all cases the interference may be eliminated by conversion of the carbonyl compounds to the cyanohydrins prior to the titration. Table III shows data obtained by direct titration using methanol as solvent and taking the first sign of an end point, using pyridine as solvent, and employing the cyanohydrin technique which represents the actual water content.

Aldehydes, as exemplified by butyraldehyde, tend to give low results when pyridine alone is used as solvent. Apparently, a reaction occurs between pyridine–sulfur dioxide and water.

Quinone is reduced by the hydriodic acid always present in Fischer reagent, liberating a molar equivalent of iodine. Pyridine is of no help. The cyanohydrin method is unsatisfactory, at least for the visual titration, because of extreme darkening of the solution. Possibly electrometric titration will be applicable.

Amines in alcohol solution, stronger than pyridine, tend to interfere primarily by obscuring the end point. This interference is eliminated effectively by use of acetic acid as diluent.

Mercaptans (thiols) are oxidized quantitatively by the iodine of Fischer reagent.

$$2RSH + I_2 \longrightarrow RSSR + 2HI \qquad (9)$$

This interference can be eliminated by prior addition of the mercaptan to an active olefin (*18*).

$$RSH + R'CH{=}CHR' \xrightarrow{BF_3} R'CH(SR)CH_2R' \qquad (10)$$

Xanthates, because of the ease with which they reduce iodine, would be expected to interfere in the Fischer reagent titration for water. However, Linch (*12*) found that free water could be determined directly in the presence of xanthates when these compounds were dispersed in chloroform. Typical results are given in Table IV.

Similar data reported by Linch (*12*) on dithiocarbamates are shown in Table V.

The analyses for water in di(2-hydroxyethyl) dithiocarbamate were obtained on samples dispersed in methanol. Chloroform was used for the other materials.

Table III. Determination of Water in Carbonyl Compounds

Compound	Environment[a]	Water Found, %
Acetone	M	0.65
	P	0.55
	C	0.50
Butyraldehyde	M	0.40
	P	0.10
	C	0.17
Cyclopentanone	M	0.45
	P	0.40
	C	0.36
Pyruvic acid	M	2.20
	P	1.45
	C	1.42
Chloral	M	0.05
	P	0.05
	C	0.04

[a] M—Methanol
P—Pyridine
C—Cyanohydrin

Table IV. Analysis of Xanthates

Compound ROCSK(NA) ‖ S	Found, Weight %		
	Xanthate[a]	Water	Total
Methyl	97.4	2.4	99.8
Isopropyl	97.3	2.1	99.4
1,3-Dimethylbutyl	96.4	4.2	100.6
Cyclopentyl	96.4	2.8	99.2
,-Ethoxyethyl	91.5	6.8	98.3

[a] By iodometric method.

Hydroperoxides react selectively with the sulfur dioxide of Fischer reagent.

$$ROOH + SO_2 \longrightarrow RHSO_4 \quad (11)$$

As no iodine or water is involved in this reaction, no interference is encountered in the titration for water. Zimmerman (*35*) published the data on aqueous hydrogen peroxide solutions shown in Table VI. Dialkyl peroxides are relatively stable and do not oxidize hydrogen iodide at a sufficient rate to offer any interference in the titration for water. Diacyl peroxides, however, are likely to interfere. The more active ones rapidly oxidize the hydriodic acid.

Water of hydration has been determined successfully on all types of compounds which offer no interference in the anhydrous state. On direct titration with Fischer reagent total water is determined—i.e., free plus hydrated. In order to differentiate, some physical separation usually must be employed, such as extraction or distillation. Typical organic hydrates which have been analyzed successfully are shown in Table VII.

Table V. Analytical Data for Dithiocarbamates

Compound R_2NCSNa ‖ S	Found, Weight %		
	Carbamate[a]	Water	Total
Dimethyl	79.0	21.2	100.2
Phenyl Ethyl	79.5	21.9	101.4
Di(2-hydroxyethyl)	98.3	1.7	100.0
	Water, Weight %		
	Added[b]	Found	
Di(2-hydroxyethyl)	0.0	1.7	
	10.4	10.1	
	15.3	15.3	
	33.6	33.6	

[a] By iodometric method.
[b] Included water originally found in sample.

Table VI. Analytical Data for Water in Hydrogen Peroxide Solutions

Peroxide, Weight %	Water Found, Weight %	Total, Weight %
30.3	69.4	99.7
20.4	80.0	100.4
14.9	85.4	100.3
6.4	93.4	99.8
3.4	96.5	99.9

Moisture in salts of organic acids in general may be titrated directly without interference. These include ammonium citrate, zinc stearate, calcium lactate, and lead acetate. Of great potential importance is the application of the Fischer reagent to the determination of moisture in penicillin sodium salt as proposed by Levy, Murtaugh, and Rosenblatt (*11*). These authors reported that the penicillin salt was thermally unstable and hygroscopic. Obviously methods suitable for this analysis were limited. Vacuum desiccation over phosphorus pentoxide was applicable but required several days. The standard ampoule was used as the flask. Platinum electrodes and buret tip were introduced through the rubber cap with the aid of hypodermic needle tips. Typical results by this rapid titration method are shown in Table VIII.

Analyses carried out under these conditions appeared reliable. As no transfers of sample were required, no opportunity was presented for the absorption of moisture from the atmosphere.

The Fischer reagent technique also is widely applicable in the inorganic field, although many class interference reactions are observed. This titrimetric method, therefore, can be employed successfully only after the chemist has become familiar with the effects of the anhydrous compounds. In general, inorganic compound reactions are stoichiometric. A point worth stressing is that materials of these types which are anhydrous or nearly so, or in which water has been determined by an independent method, may be determined quantitatively by direct titration with Fischer reagent. Thus many, but not all, metal oxides react to consume one mole of iodine per mole of oxide. Typical examples are shown in Table IX.

Table VII. Determination of Total Water in Organic Hydrates

Compound	Water Found, Weight %	Moles Water Per Mole Compound
Oxalic Acid[a] $(COOH)_2.2H_2O$	28.42	1.99
Citric Acid $HOCCOOH(CH_2COOH)_2.H_2O$	9.00	1.05
Terpin Hydrate $C_{10}H_{20}O_2.H_2O$	9.42	0.99
Dextrose $C_6H_{12}O_6.H_2O$	8.96	0.99
Chloral $CCl_3CH(OH)_2$	10.85	1.00
Cyanuric Acid $C_3N_3(OH)_3.2H_2O$	21.85	2.00
Piperazine $NHCH_2CH_2NHCH_2CH_2.6H_2O$	55.3	5.97

[a] Reference (*35*); all others (*19*).

Table VIII. Determination of Moisture in Penicillin Sodium Salt

Sample Weight, Mg.	Water Found	
	Mg.	Wt. %
466	6.08	1.30
466	6.21	1.33
227	3.57	1.57
227	3.53	1.56
484	2.52	0.52
484	2.56	0.53

All metal hydroxides studied have reacted completely, one mole of iodine having reacted for each equivalent of base.

Carbonates and bicarbonates react in similar fashion. Boric acid and its oxides first are esterified by the methanol of the reagent and the resulting water is titrated.

$$H_3BO_3 + 3CH_3OH \longrightarrow B(OCH_3)_3 + 3H_2O \quad (12)$$

$$HBO_2 + 3CH_3OH \longrightarrow B(OCH_3)_3 + 2H_2O \quad (13)$$

Cupric sulfate oxidizes the hydrogen iodide of spent reagent to form iodine.

$$CuSO_4 + 2HI \longrightarrow CuI + 0.5I_2 + H_2SO_4 \quad (14)$$

Consequently, Fischer reagent titration of the pentahydrate only shows an apparent 4.5 moles of water of hydration.

Table IX. Action of Fischer Reagent on Oxides

React Completely	React Partially or Do Not React
Calcium	
Magnesium	Aluminum
Zinc	Cupric
Silver	Iron
Mercuric	Nickel
Cuprous	Sodium
Manganese	Barium
Lead	

A few compounds, principally hydrates, have been studied which gave net apparent water values—i.e., after correction for the known water of hydration—for which no reaction mechanism was apparent. These include the chromates, dichromates, sodium sulfide, phosphomolybdic acid, zirconyl chloride, and basic aluminum acetate. All these compounds were assumed to contain the accepted water of hydration. The compositions of some, such as phosphomolybdic acid, are questionable.

An interesting means was suggested by Suter (*33*) for determining free water in some of the interfering inorganic compounds. He first separated the free water by azeotropic distillation with

xylene, after which the distillate was titrated with Fischer reagent. This technique might be improved by use of an agent, such as ethanol, dioxane, or glycol, which forms a homogeneous azeotrope with water or which boils higher than water. Then possible hold-up of water in the distillation assembly would be eliminated. Extraction also may be employed for the removal of free water from many compounds. A simple technique might involve the following: A known volume of dioxane is added to the finely divided sample. After a short contact time, an aliquant is withdrawn and titrated. Extraction, and occasionally distillation, may also serve as a means for determining both free and combined water in hydrates which do not interfere in the anhydrous state. Total water is determined by direct titration of the sample and free water by an extraction or distillation process, followed by titration.

Another variation was suggested by Rulfs (*28*) for the microdetermination of water in minerals.

The sample is placed in a combustion boat and inserted in a Pregl muffle. During ignition, moisture is evolved and condensed in a water-collecting tube at the exit of the muffle. This water is washed into the titration flask and determined by Fischer reagent titration, using the dead-stop method. Reported results are shown in Table X.

Table X. Determination of Water in Minerals

Substance	Water, Weight % Found	Calcd.
$CuSO_4.5H_2O$	36.24 36.30	36.10
$CaSO_4.2H_2O$	20.90 20.85	20.90
$BaCl_2.2H_2O$	14.75 14.70	14.75
$CaC_2O_4.H_2O$	12.22 12.29	12.32
Flint Clay (B.S. 97)	13.07 13.15	13.35
Bauxite (B.S. 69)	28.19 28.32	28.77

Table XI. Moisture in Dehydrated Foodstuffs

(Weight %)

Sample	Fischer Method	Vacuum Oven 70° C., 6 Hours	70° C., 16 Hours	100° C., 7 Hours	Air Oven, 130° C.	Toluene Distillation
Mixed vegetables	6.50	5.93	6.00	...	...	...
Carrots	6.20	4.22	5.42	...	...	...
Tomatoes	3.07	2.57	3.20	...	...	...
Protein hydrolyzate	2.44	2.48	2.41	...	...	2.40
Cornstarch	13.9	...	...	14.1	14.0	13.2

These compounds are representative of those which offer interference on direct titration or from which the water is extracted slowly and often incompletely.

Applications of the Fischer reagent to the determination of water in commercial materials, particularly natural products, appear promising. However, in many cases it is difficult to demonstrate the accuracy of the titration method because of the lack of an absolute calibrating procedure. This is particularly true of foodstuffs.

For example, consider the data reported by Schroeder and Nair (*30*) in which they compared the Fischer reagent titration, after a preliminary 5-minute extraction step using methanol under reflux, with standard AOAC methods (Table XI).

Usually the Fischer reagent results were higher than accepted procedures. However, the steady increase in the vacuum drying results from 6 to 16 hours indicated that possibly moisture was incompletely removed. On the other hand, the high values by the titration method may have been due to thermal decomposition during the refluxing step. Schroeder and Nair suggested a possibility that merits further investigation. Water of hydration may not be removed by oven drying, whereas the Fischer reagent titration will include this combined moisture. These investigators found that the water of hydration of monosodium glutamate was titrated readily with Fischer reagent, whereas little more than the adsorbed moisture was removed from this salt after vacuum drying for 16 hours at 70° C. Schroeder and Nair (*30*) observed some evidences of interference in the determination of moisture in onion powder and protein hydrolyzate, which seemed to be associated with excess Fischer reagent.

Table XII. Fischer Reagent Titration for Moisture

Method	Onion Powder	Protein Hydrolyzate
Intermittent titration	3.95	2.41
Methanol extraction (cold)	3.97, 3.93	2.56, 2.52
Methanol extraction (reflux)		
2 min.	3.90	2.47
5 min.	3.90 (3.78)[a]	2.44 (3.30)[a]
15 min.	3.84	2.51
30 min.	4.01	2.54
Excess reagent (10 to 20 ml.)		
2 hours	4.92 (10 ml.)	5.22 (20 ml.)
4 hours		5.98
19 hours	6.28	
Excess reagent		
10 ml.	6.98 (96 hours)	4.65 (4 hours)
30 ml.	7.83 (11 hours)	6.83 (4 hours)

[a] 5-Minute values for samples used in excess reagent tests.

Table XII gives a comparison of moisture values obtained under different conditions. First, intermittent titration of the sample suspended in methanol at room temperature—i.e., adjustment to the end point at timed intervals. Over periods of 6 to 12 days the values tended to increase in linear fashion. The values shown were obtained by extrapolation back to the ordinate of a plot of moisture versus time. Secondly, methanol extraction at room temperature; aliquots were removed periodically and titrated. The data represent total water found, using methanol originally containing 0.03 to 0.06% and 0.54% water, respectively. Methanol refluxing showed little variation. However, when excess reagent was allowed to stand with the sample, extremely high values resulted. Ascorbic acid is the only compound reported in dehydrated foods which would cause high values. However, Johnson (*8*) demonstrated that the quantity of ascorbic acid found in these foodstuffs was equivalent to no more than 0.03% water.

Further investigations should include a more thorough study of the effects of particle size on "apparent" moisture recoveries. More work on extractions employing the Waring Blendor or a modification should also aid in ascertaining the nature of the apparent interferences. Finally, the compositions should be studied of these erratically behaving compounds in order definitely to ascertain potential interferences.

McComb's study of several protein materials showed no wide variations, even though excess Fischer reagent was in contact with the sample for periods up to 3 hours (*13*). Brobst (*1*) employed the Fischer reagent titration successfully for the determination of moisture in lecithin and crude soybean oils.

Several interesting applications of the Fischer reagent titration method have been reported for the determination of moisture in polymers and their intermediates. Many of these materials are thermally unstable and, therefore, cannot be analyzed by oven drying or azeotropic distillation methods. For such chemicals the Fischer method may be the only feasible technique available. In order to apply this titrimetric method safely, however, a thorough knowledge of potential interferences must be obtained.

Formaldehyde in aqueous solution or in any of its polymeric forms does not interfere. However, water in dimethylol urea or other low molecular weight polymers of formaldehyde and urea cannot be determined directly at room temperature, presumably because of further condensation of the polymer or condensation with the methanol of Fischer reagent. In either case water would be formed. Only at a temperature of about −40° C. does this interfering reaction become sufficiently slow to permit direct titration for free water originally present in the sample (*19*). On the other hand, urea-formaldehyde resins may be titrated at room temperature. Cornish (*3*) demonstrated that this and several other molding powders could be analyzed successfully. His technique usually involved dispersing the sample in meth-

anol, refluxing for a short time, and titrating the cooled heterogeneous mixture. Typical results are shown in Table XIII.

Where possible, the data were checked by an azeotropic distillation procedure, which gave results within 0.1 to 0.2% of those found by the more rapid titration method.

Other successful applications include the direct determination of moisture in polythene (*23*), vinsol resin and rosin size (*14*), shellac-alcohol solutions (*27*), paints and varnishes (*34*), and synthetic rubber (GR-S) (*29*). While developing his technique for the analysis of paints and varnishes, Swann (*34*) reported that zinc oxide was the only powdered pigment found which interfered in the titration.

Rush and Kilbank (*29*) found that a method employing Fischer reagent titration was the only satisfactory technique for the determination of moisture in GR-S. They covered the sample with benzene, refluxed, added ethanol, and distilled the homogeneous ternary: water-benzene-ethanol. The distillate was titrated with Fischer reagent. Moisture found in compounded GR-S stock varied from 0.042 to 0.520%.

The determination of moisture in explosives represents another field in which the Fischer reagent titration should find wide application. Typical applications are shown in Table XIV.

Further investigations should be made in this field. For example, when trinitrotoluene was dispersed in methanol, the mixture formed a deep red color during titration with Fischer reagent which obscured the visual end point (*26*). An electrometric method should be tried to determine whether an interfering reaction is involved. Certainly one would not predict any abnormal behavior.

Applications of the Fischer titration method to analyses for moisture in petroleum products have been covered adequately in the literature (*20*). Satisfactory techniques have been reported for condensable and noncondensable gases employing extraction by methanol at −78° C. Direct titration methods are commonly employed for many liquids and solids, either as homogeneous solutions in an inert solvent or after liquid-liquid extraction into an immiscible liquid. These titrations are made most easily in the presence of sufficient alcohol or pyridine to assure a solvent for the Fischer reagent end products. Otherwise, false end points may be encountered.

In applying the titrimetric method to carbohydrates, the absolute accuracy often is unknown because of the lack of a suitable reliable standard method. This applies particularly to the starches which normally are analyzed by oven-drying techniques. Literature reports have given conflicting conclusions, but for the most part have indicated that Fischer titration is reliable provided a preliminary extraction step—e.g., cold or hot extraction with methanol—is employed. Applications to sugars (direct titration), paper, and fabrics (extraction before titration) have been adequately demonstrated (*22*). At least one extensive study has been reported on the determination of moisture in native wood (*15*). Room temperature methanol extraction effected about 96% removal of moisture from small pieces of cypress, Douglas fir, or oak in 1 hour and up to 98.5% in 3 hours. More rapid extractions were feasible for the determination of moisture in wood pulp (*14*) and sawdust (*27*). Preliminary data indicate that wool may be analyzed rapidly and precisely. In one series of experiments, employing a 30-minute methanol extraction at room temperature before titration, a sample of wool analyzed 9.30 ± 0.01% compared to 9.26 ± 0.08% found by oven drying at 102° (*21*). A comprehensive study of the Fischer reagent titration for moisture in wool would be desirable, as a time-saving and reliable procedure should result.

The Fischer reagent should be useful for the determination of moisture in soaps and soap products. Values obtained on direct titration would be subject to a mole for mole correction for any free alkali present. Normally this correction would be small. In one reported study (*26*) Ivory flakes were dispersed in methanol and titrated immediately with Fischer reagent. Values of 3.02 ± 0.02% moisture compared favorably with a result of 2.99%, obtained by azeotropic distillation.

An electrometric study of direct titration for moisture in coal might result in a rapid routine analysis.

This discussion of necessity has covered only a portion of the applications, real and potential, of the versatile Fischer reagent. Obviously in many specific cases more rapid techniques are available, but no procedure approaches this titrimetric method in general applicability. Certainly the Fischer reagent should be considered in all studies involving the determination of water.

Table XIII. Moisture in Plastic Molding Powders

Material	Water Found, Weight %
Urea formaldehyde	11.6, 11.6, 11.7
Phenol formaldehyde	4.85, 4.83
Acetyl cellulose acetate	1.81, 1.80
Cellulose acetate	0.24, 0.25, 0.26
Polyvinyl alcohol	1.82, 1.81

Table XIV. Moisture in Explosives

Material	Water Found, Weight %: Fischer titration	Water Found, Weight %: Other method	Reference
Nitrocellulose	2.8 ± 0.0	2.9[a]	*14*
Nitroglycerin	0.285 ± 0.005	0.29[b]	*14*
	0.08 ± 0.00	0.08[b]	*24*
Gunpowder (grained)	0.945 ± 0.005	1.03[c]	*27*
Priming explosive	12.4 ± 0.1	11.9[c]	*25*
Smokeless powder	0.27–0.29	0.265–0.285[b]	*5*

[a] Azeotropic distillation. [b] Desiccation. [c] Oven drying.

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Electrical Measurement of Water Vapor with a Hygroscopic Film

E. R. WEAVER, *National Bureau of Standards, Washington, D. C.*

Measurement of the electrical conductance of a film of a hygroscopic electrolyte provides one of the easiest and most sensitive methods of determining water vapor. The development of the method from its beginning in 1918 is described. In its earliest forms the method was unsatisfactory because the conducting films did not retain constant calibrations. Dunmore succeeded in preparing films of lithium chloride in polyvinyl alcohol on a polystyrene base. These films have satisfactory stability, are commercially available in convenient form for measuring relative humidities above 5%, and are easily adaptable to recording and control. Much more sensitive films of sulfuric and phosphoric acids permit more rapid determinations of much lower concentrations of vapor in very small samples, but require calibration at the time of use. The construction and uses of the hygrometers of both types are described.

THE electrical method of measuring water vapor had its inception in 1918 as a proposed means of measuring the dryness of gases in synthetic ammonia plants. Before the organization of the Fixed Nitrogen Research Laboratory in the Department of Agriculture, the development of methods of analytical control for the projected plant at Muscle Shoals was assigned to the Bureau of Standards. Some of the catalysts proposed for use in the process were rapidly poisoned by water, and completeness of drying was a major factor in its successful operation.

The only quick method then known for determining water in as low a range as desired was the measurement of the frost point. As the saturated solution of even a slightly soluble electrolyte has a vapor pressure lower than that of pure water, and water will begin to condense when the vapor pressure of a saturated solution is reached, it was obvious that, down to its eutectic point, any fairly soluble electrolyte would condense water, at a given temperature, from a drier gas than would produce frost. The electrical conductivity of the solution formed gives a measure of the water condensed. It was quickly learned, with a little experimentation, that the more hygroscopic salts and alkalies could thus be used to detect amounts of water less by a factor of 4 or 5 than could be detected by the deposition of dew or frost at the same temperature, but that with phosphoric acid the factor was at least two and with sulfuric acid three orders of magnitude.

The first detector consisted of two wires wrapped close together around a glass tube and coated with a solution of calcium chloride. The resistance between the wires was a measure of the humidity. This device was quickly superseded by a platinum-coated glass tube, around which a fine line was scratched or etched to separate the metal into two electrodes. It was coated with a solution of sulfuric acid, to which gelatin was added.

An alternating current galvanometer was used in a Wheatstone bridge for measuring the resistance of the detector. This instrument was insensitive, as compared with later instruments, and it was necessary to employ relatively long and narrow gaps between the electrodes and a relatively thick coating of electrolyte to produce readable deflections of the galvanometer. It was a great advantage that the water to be measured was concentrated by the high pressure existing in the ammonia plant, and investigators were overly impressed by the ease of applying the detector to a closed system without withdrawing or contaminating the sample. The title "Detector for Water Vapor in Closed Pipes" was therefore attached to the first paper (*8*) describing the method, although its general applicability was recognized.

Early calibrations made with the vapor from known solutions of sulfuric acid and water showed that just after calibration small concentrations of water vapor could be determined with an ease and precision incomparably better than any other method with which the investigators were acquainted. But none of the hygroscopic films would hold a calibration long enough to justify the work of calibrating, except for a few special purposes. It was found useless to make a determination of water vapor in gas that had passed through even the little rubber tubing needed to make a close end-to-end connection between glass. Many years later it was discovered that the film of oil left in drawing copper tubing can also be a troublesome reservoir of dissolved water, unless the oil is driven out before the apparatus is assembled.

The control of the synthetic ammonia plants was taken over by the Fixed Nitrogen Research Laboratory, and for the next 15 years the electrical method was used for water at the Bureau of Standards only once or twice a year, and then usually only as a qualitative detector, to show that some gas had or had not been satisfactorily dried. Possibly the most useful purpose it served was to convince a metallurgist that he could not keep hydrogen free of oxygen if he passed it through tubing connected with rubber. The test was accomplished by passing the contaminated hydrogen through a small combustion tube before it reached the hygrometer.

This was the first example of a use for which there are many potential applications, that of a quick, sensitive, and easily applied detector of oxygen in hydrogen or of hydrogen or any of its compounds that can be burned in air.

DUNMORE HYGROMETER

In 1935, the author tried and failed to make an instrument satisfactory for exploring the moisture content of the upper atmosphere. The failure resulted from the old difficulty of finding a film that would hold a calibration. Dunmore succeeded in solving this difficulty (*3*, *4*). In the development of instruments for meteorological work and for laboratory and industrial applications, he had the cooperation of the Friez Instrument Division, Bendix Aviation Corp., Baltimore, and the American Instrument Co., Silver Spring, Md., respectively. Hygrometers of the Dunmore type are obtainable from these manufacturers and from one or two others who entered the field later. The hygroscopic film with its electrodes and supports as made by the American Instrument Co. is called a sensing element; in combination with the necessary electrical equipment, it is called the Aminco-Dunmore hygrometer; and its combination with an enclosure in which the sample to be analyzed is confined, is referred to as a "hygrocel."

In its present form the Aminco-Dunmore sensing element consists of a hollow cylinder of polystyrene on which two fine palladium wires are wound close together. The cylinder is dipped into a solution containing either lithium chloride or lithium

bromide as a hygroscopic electrolyte and polyvinyl alcohol, which acts as a mechanical support and serves to prevent crystallization of the salt. The hygrometer is seasoned for 15 days at constant temperature and a humidity near that of a saturated solution of the salt.

It is calibrated by the manufacturer at numerous temperatures and at the range of relative humidity within which its auxiliary electrical instruments give satisfactory readings. Because the change of resistance produced by a moderate change of humidity is enormous, the suitable range of humidity of any one element is rather narrow, and to meet this difficulty the sensing elements are made in eight ranges. They differ principally in the concentration of the dissolved electrolyte, but also in whether chloride or bromide is used—for example, at 80° F., the first element of the series has a calibrated range of relative humidity from 5 to 15%; the eighth, from 82 to 98%. For special purposes the sensing elements have been used to measure relative humidities as low as 1.5%. When wider ranges of humidity must be measured, an ingenious combination of several sensing elements with metallic resistors devised by Dunmore is employed, not only to extend the range but to give an over-all calibration curve that is nearly linear. The sensing elements may be applied to instruments for electrical recording and control as well as for simple indication.

The sensing element works at a relative humidity far below that at which the electrolyte it contains, when alone, becomes a nonconducting crystal. The ions in their bed of hard plastic evidently retain enough mobility to conduct current in the presence of moisture, but not enough to get together into the nonconducting crystalline form. If a sensing element is kept very dry or very wet for too long a time, its stability may be lost, however, always with an increase of resistance.

The Aminco-Dunmore hygrometer can be used to give a continuous indication of the moisture content in any gas phase, with only a few minutes' lag, if there is nothing present that will damage the sensing element. Concentrations approaching saturation of any of many organic substances would soften one or the other of the plastics used and ruin the calibration. The hygrometer is regularly calibrated over the range of temperature from −20° to 140° F. The sensing elements can be handled without danger of damage and plugged like a radio tube into a standard socket.

The potential applications of such a hygrometer are many. Its most interesting use is probably that for which it was first developed. Attached to a balloon it not only "senses" the humidity of the upper atmosphere but, by controlling the frequency of an oscillating circuit, it tells by radio what it has found. Its use for controlling equipment for air conditioning or industrial drying is obvious. Less so, perhaps, is the fact that at cylinder-charging pressures the instrument is sensitive enough to indicate the moisture in commercial "dry" nitrogen or oxygen before the permissible limit is reached. Its largest field of potential application is probably in determining the strength of concentrated solutions or the moisture content of solids for which a reasonably constant relation can be worked out between the water in the solid and in the air in equilibrium with it. This has been done for such diverse materials as whole-kernel corn (*2*), shredded coconut, a cattle food made from brewery waste, alum, kaolin, paper, sawdust, leather, and molding sand ready for casting (*5*). In the case last mentioned, an open-sided hygrocel is simply placed in contact with a flat surface of the mold. In most other cases a sensing element is placed in a storage bin or in a conveyor.

WATER VAPOR INDICATOR

As World War II approached, the armed forces believed they were losing airplanes through the freezing up of oxygen regulators and later through the freezing of carbon dioxide equipment used for fighting fire or operating mechanical controls. The trouble usually occurred, not because the gases put into the cylinders were not dry, but because the water was in the cylinders themselves. Probably it was usually adsorbed by the rust on the cylinder walls, but sometimes the water was introduced in the suspension of graphite and water or even the edible southern cane molasses used to lubricate and seal the valve threads. It became necessary to test each cylinder delivered for service.

Until this situation arose, all efforts to solve the problem of changing calibrations of the "sensing elements" or "detectors" had been directed toward making the detectors themselves sufficiently stable to permit the use, over a reasonably long time, of a somewhat laboriously determined calibration curve. It now seemed desirable to take advantage of the much more sensitive films of phosphoric or sulfuric acid and to avoid the difficulty of stabilizing them by calibrating them at the time, temperature, and water concentration at which they were used by matching their readings with those of an atmosphere, the water content of which could be inferred from other measurements.

The best method of doing this seemed to be to change the pressure of a stream of gas of known water content until the reading of the detector was duplicated. The known water content could be obtained either by saturating the standard ("comparison") gas at high pressure or by introducing approximately the desired amount of water into a cylinder to be used as a secondary standard and determining it by comparison with gas from a saturator. For example, when the air from a saturator at 100 atmospheres' pressure is reduced to 1 atmosphere, it has a relative humidity of approximately 1%. If gas from the cylinder used as a secondary standard matches, at 100 atmospheres, the gas from the saturator when reduced to 1 atmosphere, the secondary standard at 1 atmosphere has a relative humidity of 0.01%.

There was a great deal of trouble with fancy saturators at the start. The saturator now recommended is a pair of small high-pressure cylinders connected in series, filled nearly to the neck with coarse sand, and with 2 or 3 inches of water in the bottom of each, through which the gas has to bubble. Both cylinders should be in the same bath. Gas bubbling through the water in the first cylinder becomes almost saturated at the temperature of the bath, but in the middle of the mass of sand probably causes a little cooling, the amount of which is not easy to measure. Because the gas is already in almost complete equilibrium with water, it evaporates almost no water in the second cylinder, the temperature of which remains that of the bath. The outlet valve may be wound with a small electric heater to prevent condensation of the water by the cooling of the valve by expansion. Checks by independent methods have shown a very close approach to saturation in the first cylinder. None have been made on the output of the second, because it is considered more reliable than any method that might be used to check it.

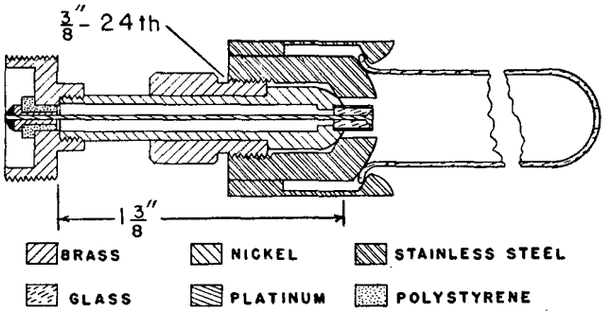

Figure 1. Water Vapor Detector with Test Tube Holder

The detector now recommended differs from the preferred one described in earlier papers (*9*, *10*) in two respects. Instead of being made in the form of a screw plug, it is a union similar in form to a miniature connector to an oxygen cylinder valve (Figure 1). The body is made of nickel, and the platinum tip is gold-soldered into it. This permits the central electrode to be sealed in glass or removed and replaced without damaging the detector otherwise. This was not possible with the plugs formerly used. The change from a plug type to a union type of fitting was made to facilitate shifting the detector from one piece of apparatus to another.

The electrical measuring instrument previously described is still used with satisfaction, although its electronic amplification is low. Unfortunately, there was an error in the wiring diagram

first published. The corrected diagram is shown in Figure 2. Much higher amplification is practicable, but seems to be unnecessary because the precision of the measurements is limited by the pressure gages rather than by the detector or its electrical connections, and the ultimate sensitivity is limited by the interference of water from extraneous sources, which is extremely difficult to avoid. Actually in this method as ordinarily employed, the detector is simply a null-point instrument, as is a galvanometer in a potentiometer circuit. The measuring instruments are pressure gages, just as the measuring elements of the potentiometer are adjustable resistances.

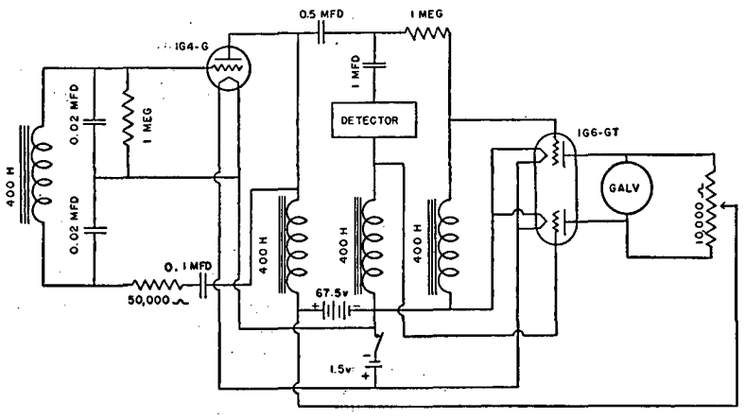

Figure 2. Electrical Circuit

The extraneous water comes from many places which must be hunted down in the individual equipment. Pressure gages and valve packings have caused the most trouble, always excepting rubber. The trouble from valve packings seems to have been almost or entirely eliminated by using Teflon, which is easily made tight, produces little friction, and seems to retain no water. Soldering flux, or glycerol, used in making a litharge joint absorbs water or gives it off, depending on the gas with which it was previously in contact, and the surface oxides on all metal parts tend to do the same. They act as a trace of silica gel might be expected to do. Iron rust is the worst, but there is some indication of trouble from the oxides on brass and even aluminum. However, only with very small samples of extreme dryness are these oxides troublesome.

It is difficult to free gages of water. Baking them and repeatedly evacuating them while hot have seemed to eliminate trouble for a time, but it always comes back, probably because hygroscopic materials are not completely removed. A gage should always be on the downstream side of the detector in all critical measurements. Glass sometimes causes a little trouble, but less than might be expected from all that has been said by workers in spectroscopy and electronics about its "adsorption" of water. Its slowness and large magnitude would indicate that much of the pickup of water on glass is solution and slow diffusion into the extremely viscous liquid rather than a surface phenomenon.

The solution of the electrolyte in the glass of the detector is unquestionably one of the hardest interfering effects to deal with. It results in slightly conducting layers under the main conducting film that reach equilibrium with extreme slowness and account for most of the annoying slow drifting of readings. It can be largely eliminated by washing and drying the detector when not in use and honing the detecting surface with a little fine abrasive when drifting becomes too bad. The concentric electrodes and the glass between them should be made longer than in the past, both to permit this grinding away and to minimize the danger of failure under high pressure. A length of 1 cm. is suggested.

The change of calibration of detectors of this type is more the result of reaction with and solution in the glass than of evaporation of sulfuric or phosphoric acid, to which it was first attributed. Substituting polystyrene or other plastics for glass makes the apparent solution of the electrolyte worse rather than better.

In working with gases containing more than about 10 micrograms of water per liter, phosphoric acid is the most satisfactory hygroscopic electrolyte. It does not work much below that concentration, probably because of the formation of a crystalline phase. The author has found no limit below which sulfuric acid is not conducting and responsive to changes in the vapor pressure of the water above it, but the films are much less stable than those of phosphoric acid. Accordingly, the use of a mixture of phosphoric and sulfuric acids in the proportion of about 4 to 1 is recommended. This seems to avoid the insensitivity of phosphoric acid without sacrificing much of its stability.

As compared with the use of the Amineo-Dunmore sensing element, the method has the serious disadvantage of always requiring the presence of an observer and some manipulation. It is not easily made recording. On the other hand, it can be used in very small space, a fraction of a cubic centimeter if necessary. It is much more sensitive than the Dunmore element and has a much quicker response, which makes it possible to test a succession of samples in less time as well as to follow more rapid changes in an atmosphere. Its range of resistance, its speed, and its sensitivity can be changed at will and without loss of time. It is not subject to damage by high concentrations of organic vapors. The acids used are neutralized by ammonia or vapors or dusts of other alkaline materials; this seems to be about the only chemical effect to be looked out for.

Bartlett (*1*) and others have established that the concentration of water vapor in equilibrium with liquid water always increases with the pressure of another gas in the gas phase, with the exception of hydrogen and helium at moderate pressures. The effect of this on the indications of the detector is in the opposite direction to the effect of deviation of the noncondensable gas from Boyle's law, is of much greater magnitude, and must be corrected for.

If the effect did not exist, and if the gas obeyed Boyle's law exactly, we could write

$$C = \frac{SP_c}{P_s} \tag{1}$$

where C is the concentration of water vapor at pressure P_c, S is the concentration of water vapor at saturation—that is, in equilibrium with liquid water—and P_s is the pressure of saturation before expansion to P_c. By using an empirical formula of approximation of the usual type, the deviation from the ideal of both permanent gas and vapor may be taken into account by the convenient equation

$$C = \frac{SP_c(1 + kP_c + k'P_c^2 + \ldots)}{P_s(1 + kP_s + k'P_s^2 + \ldots)} \tag{2}$$

Below a pressure of 100 atmospheres, which is about the maximum usually involved in the use of the indicator, $k'P^2$ and succeeding terms may be neglected for most work, and the coefficient, k, is found to be a negative number. Hence, substituting $K = -k$ so that the symbol will represent a positive integer,

$$C = \frac{SP_c(1 - KP_c)}{P_s(1 - KP_s)} \tag{3}$$

The process involved in the determination of the concentration, W, of water vapor at pressure P_w of an unknown gas is to

adjust the pressure of the gas until, at P_x, the gas has the concentration C.

Then

$$C = \frac{WP_x(1 - KP_x)}{P_w(1 - KP_w)} = \frac{SP_c(- KP_c)}{P_s(1 - KP_s)}$$

and

$$\frac{W = SP_cP_w(1 - KP_c)(1 - KP_w)}{P_sP_x(1 - KP_s)(1 - KP_x)} \quad (4)$$

which is the equation for general use with the detector.

In order to establish the value of K for oxygen, the water was measured in a cylinder of compressed oxygen both by means of the electrical indicator and by weighing. The value of K can be determined either by accepting the concentration of water vapor found gravimetrically and solving for K in the case of one or more electrical tests, or from the electrical tests alone by matching the gas of unknown water content against gas expanded after saturation at various pressures and substituting a value of K which will give the most consistent values for the vapor content of the expanded gas.

In the case mentioned, there were three gravimetric tests, each of which employed about 300 liters of sample and required about 24 hours for completion, and 22 electrical tests which required only 2 or 3 hours and perhaps 10 liters of gas. Comparing the average of the electrical tests with those of the gravimetric tests led to a value of K of 0.00015 when pressures were expressed in pounds per square inch (this is the most convenient unit because it is the one in which commercial gages are usually graduated).

Comparing the electrical tests by a graphic method only among themselves indicated a slightly higher value, on the basis of which the author predicted (*10*) that the value $K = 0.00017$ would eventually be found the more accurate. Subsequently, Howerton (*7*) with the aid and the laboratory facilities of the Chemistry Department of the University of Maryland made a careful gravimetric determination of the effect of the carrying power of oxygen for water vapor at pressures in the approximate range of 0 to 100 atmospheres and arrived at the value $K = 0.00017$.

With data expressed in a somewhat different form, the correction for the much larger effect of carbon dioxide pressure derived from electrical measurements at different pressures was in accurate agreement with the studies of Wiebe and Gaddy (*11*) of the system water–carbon dioxide. These two gratifying agreements with the experimental work of others do not demonstrate that the use of the detector is a superior method for measuring the effect of the presence of other gases on the equilibrium content of water vapor. It does demonstrate that, for use in computing analyses, values of K may be trusted if they are derived only from the effect of changing the test pressures of the gas being analyzed.

The water vapor indicator, a name used to distinguish it from the Dunmore hygrometer, has been very successful for the purpose for which it was originally designed, the measurement of water in compressed gases. If other people change the cylinders, one observer with one instrument can readily test 100 oxygen cylinders per hour for compliance with the specification that the gas first delivered contain not more than 20 micrograms per liter, and with no uncertainty that every cylinder meets the specification. Not enough gas will be removed from any cylinder to be detected with an ordinary pressure gage.

Second in extent has been the use of the indicator to test the dryness of refrigerants, particularly the Freons and other halogen compounds. As these materials are commonly shipped as liquids nearly filling their containers, we are primarily interested in the concentration of water in the liquid phase; but the instrument works only in the gas phase. Fortunately, according to Henry's law the relative concentrations of water in gas and liquid phases are constant. While Henry's law may be no more exact than the other laws which describe the ideal state, deviations are insignificant when the solubility in the liquid phase is slight, as is the case with most liquefied gases. Deviations might be expected to be large in the case of ammonia and some other gases.

Howerton (*6*) has described a straightforward method of measuring the partition coefficient or Henry's law constant by using the indicator to measure the water, first in the gas phase in equilibrium with the liquid, and then in vapor produced by vaporizing completely a small sample withdrawn in the liquid phase. He has determined the coefficient for numerous substances over a considerable range of temperatures and found that results satisfactory for ordinary purposes can be obtained without serious difficulty. Once the partition coefficient has been determined, the water content of the cylinder as a whole can be quickly and easily found by a single observation, with the detector, of the water vapor in the gas phase. Because the composition of the gas and liquid phases changes during distillation, the smallness of the sample is a great advantage in this case.

The potential applications of the vapor detector in the laboratory are to be inferred from its properties rather than described in terms of experience, which is still limited. Chief of these properties are high sensitivity, wide range, speed, simplicity of construction and use, specific indication of water without interference by many commonly encountered gases or vapors, and small size of both the detecting element and the sample needed. Because the testing and calibration are usually done within a few minutes of each other, the effects of temperature or of changes in the electrical instruments are eliminated with changes in the detector itself.

The indicator can be used with ease and precision to follow the evolution of water resulting from physical or chemical change—for example, to follow the dehydration of silica gel at elevated temperatures with a sample of less than 1 gram.

The use of the device as a sensitive detector of substances that are easily converted into water, such as the hydrocarbon gases in air, has been mentioned. The ability to measure minute amounts is about the only thing needed for determining permeabilities of films to gases and vapors. The determination can be made in either of two ways. The membrane may be clamped between the halves of a "cell," in one half of which is the gas or the saturated vapor to be tested, in the other the detector and a hot wire or other means for producing combustion. The space in which the detector is located is swept out with a stream of dry air rapidly enough to prevent an appreciable concentration of vapor and long enough to satisfy the observer that the film has been satisfactorily "conditioned." Flow is stopped, and the time needed to build up a definite concentration of vapor in the dry space is observed. The serious difficulties and sources of error in the method are connected with the condition of the membrane, particularly with the internal gradient of the substance diffusing through it rather than with the method of measurement. The control of temperature is very important in this operation.

A relative humidity of 10% builds up in a 1-inch space on one side of ordinary cellophane with saturated air on the other side, in about 20 seconds. With a film of saran of the same thickness, about 40 minutes are required.

Probably, more accurate results are to be obtained, particularly when combustion is involved, if the space into which diffusion is taking place is swept out continuously with a measured stream of dry air or, when permeability to oxygen is being determined, in a stream of dry hydrogen. If permeability to other things than water is being determined, the gas is passed through any convenient device for producing combustion and then over the detector. The testing of a flowing stream rather than a static atmosphere permits the rate of penetration of film to be followed from its beginning to the establishment of a steady state without much change in the conditions on the two sides of the membranes. The static method should be applicable to samples of membranes

only a few millimeters across. The flow method would hardly serve this purpose.

WATER-VAPOR PRESSURE OF LIQUIDS AND SUGGESTED APPLICATIONS

A type of application that is almost wholly unexplored is determination of water in solution in various liquids by measuring the water in the gas phase in contact with it. The apparatus required is of the utmost simplicity.

The detector is merely screwed into a holder for a small glass tube with a hole at the lower end and another on the side near the upper end. The tube with the detector in place is dipped into the liquid and allowed to fill until the side outlet is covered. As the liquid enters the tube well below the surface, the otherwise troublesome effect of prompt solution in the surface layer of water from the air is almost entirely avoided. A steady reading is obtained, usually in 2 or 3 minutes, and can be repeatedly checked. It is probably most convenient to put this reading in terms of relative humidity in the vapor phase, because this figure is much less affected by temperature than is absolute humidity.

The equilibrium humidity is very different for the same water content in various liquid compounds, and unfortunately the relation between the two seems never to be the direct proportionality to be expected from Henry's law. The curve for ether, for example, instead of being a straight line is as perfect an integral sign as could be made by the most artistic professor of mathematics. However, once the curve has been determined for a pure compound, the water content of a reasonably pure one can be determined quickly with a minimum of effort and with considerable precision. The first application of the method is obviously to the determination of the dryness of reagents or "pure" compounds. The second is to the determination of surface moisture in an inert material, say wet sand, a small sample of which can be shaken with a liquid such as sulfuric acid or alcohol. The resulting solution can be tested immediately without removing the solid. This method can probably be applied to the determination of the moisture held by capillarity or surface adsorption in most soils and many commercial products. The more gradual extraction of more firmly held or combined water can be followed if desired. As water solvents, sulfuric acid, alcohol, acetone, ether, carbon tetrachloride, or even a hydrocarbon can be used, depending on the nature of the material to be extracted and the amount of water to be expected. Generally the best results are obtained if the relative humidity of the vapor of the resulting solution is below 50%.

This method may be applicable to determining water in butter, soaps, lubricants, bituminous materials, and emulsions of various kinds. The material to be tested should be dissolved in or extracted with a solvent, perhaps ether for a fat, alcohol for a soap, candy, or a watery emulsion, benzene for tar. All these tests will unquestionably be complicated by the fact that ideal solutions of such materials are rarely available, but many of them may not be too far from ideal to permit satisfactory corrections for the effects of the dissolved substances. These effects can be found approximately by comparing a few samples of known water content with similar amounts of water in the pure solvent.

The solvent need not necessarily be a single compound. The disadvantage of using ether, benzene, or carbon tetrachloride as a solvent is that except for very dry substances the sample must be excessively small or the water will separate in another liquid phase. On the other hand, alcohol is a poor solvent for many things and is relatively insensitive because a large amount of water is required to change its vapor pressure much. A mixture of a good solvent for the class of materials being analyzed and a good solvent for water may be found advantageous and, if used in constant proportion, should be no harder to adapt to an analytical procedure than a single solvent.

Any analytical procedure based on the proposed method will require much patient work, but perhaps less than might be supposed; once known materials with known water contents are available to work with, observations can be made with great rapidity. The method may be expected to have the sensitivity, speed, simplicity in use, and applicability to small samples characteristic of the electrical method in other applications.

Another application offers interesting possibilities—for instance, the determination of the proportions in which two solvents are mixed

An unknown mixture of ethyl ether and ethyl alcohol contains enough water to produce a relative humidity of 20% in the vapor. If the liquid is otherwise pure alcohol, it contains about 2% of water. If it is pure ether, it contains about 0.85% of water. Now 0.5% of water is added. If the sample is alcohol, the relative humidity of the vapor will increase about 2.2%; if it is ether, it will increase about 55%. If the system has been worked out experimentally, it should be possible to interpret an intermediate value in terms of the amount of each of the three constituents.

Available data on the relations between the concentrations of water in vapor and liquid phases show curves of surprisingly different forms, some of which may have theoretical interest. The differences among sulfuric acid, nitric acid, and acetic acid are striking. Only less so are the differences among alcohols, ketones, esters, ethers, and hydrocarbons.

The indicator may not find extensive application to the determination of substances other than water, unless water is to be determined at the same time. When suitable apparatus is at hand, density and refractivity are even easier to measure than water vapor; but the feasibility of measuring the water vapor adds another to the list of quick and fairly sensitive physical methods of analysis which have enough possibilities to justify mention.

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[END OF SYMPOSIUM]

Mass Spectrometer Analysis of Some Oxygenated Compounds

The Sorption Problem

HAROLD M. KELLEY[1]

Polychemicals Department, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

The versatility of the mass spectrometer was increased by heating the inlet tube. This extended the boiling point range of sample components because it reduced sorption in that critical tube. Various oxygenated organic compounds, most of which contained an OH group, were studied to define the cross contamination caused by sorption. It was found that some extremely complex mixtures of oxygenated compounds could be analyzed with satisfactory precision. Using isomeric nonyl alcohol mixtures (boiling point about 200° C.), samples were compared for similarity of composition only. Isomeric analysis was not possible because pure standards were unavailable. Some of the limitations of the analysis of unknown oxygenated mixtures are discussed. It is suggested that plant control analyses need only show that the process is within control limits—component concentrations are not always required. Similarity of control mixtures and process samples as shown by their spectra should often provide sufficient control information so that detailed analyses are not necessary.

THE application of mass spectrometry to the analysis of hydrocarbon mixtures has been well demonstrated (*2*, *13*). Of especial importance is the ability to resolve mixtures containing isomeric hydrocarbons. In industrial research, complex mixtures of homologous series of a given class of oxygenated organic compounds boiling over a range of several degrees are commonly encountered, and often more than one class of compounds may be present. Mass spectrometric methods for analyzing some mixtures of this sort have been described (*9*). The difficulties encountered were due principally to sorption of certain components on the walls of the gas inlet system, so that correction factors were required. Polar compounds, such as those containing a hydroxyl or amine group, were the most strongly sorbed and in general the degree of sorption increased with molecular weight. Techniques by which sorption can be reduced were developed in several laboratories (*5*, *6*, *8*, *12*). One technique involved heating the inlet tube between the leaks and the ionization chamber to 200° to 250° C. (*4*).

The work reported here was done with oxygenated compounds using a heated inlet tube. The objective was to ascertain the agreement that could then be obtained between mass spectrometer results and synthetic mixture compositions following the standard schedule for liquid hydrocarbons. The resulting analyses indicate that sorption need not create as much difficulty as is commonly believed.

APPARATUS AND TECHNIQUE

This work was started on a Consolidated Engineering Corp. mass spectrometer 21-101. The operating conditions were an ionizing current of 47 microamperes, an ionizing voltage of 50 volts, and a magnetic field current of 703 ma., by means of which *m/e* 42 was in focus at an ion-accelerating potential of 1650 volts.

The instrument wasc onverted to a C.E.C. 21-102 before the alcohol-ester mixture was analyzed and the nonyl alcohol comparisons were made. Operating conditions then were a catcher current of 9.0 μa., an ionizing voltage of 70 volts, and a magnetic field current of 703 ma., by means of which *m/e* 42 was in focus at 1650 volts. The ionization chamber temperature was 240° C.

To minimize sorption, the inlet tube on C.E.C. 21-101 was heated to 240° C. by means of Nichrome ribbon wound on a single layer of $^1/_{16}$-inch asbestos paper between the leaks and the glass cover plate. A copper-constantan thermocouple was used to measure the temperature. Finally two layers of $^1/_{16}$-inch asbestos and a layer of aluminum foil were wound about the tube. When the instrument was modified to C.E.C. 21-102, a section of heater was bifilar wound directly on the glass between the ring seal at the glass ground joint and the precision tubing; a glass hook sealed near the precision tubing held the bight. Tungsten seals through side arms at the ring seal were used to connect the internal heater to the external circuit. This arrangement required water cooling of the metal cover plate. Because water failure could lead to overheating of the cover plate wax seals, a switch was arranged so that the internal heater was operated only during analysis periods.

All samples were pipetted into the inlet volumes with a micropipet through a mercury-covered sintered disk (*11*). The pipet bore was calibrated with mercury. Gas pressures in microns in the inlet bottle were calculated from the pipetted volume and the gas law.

All samples flowed through the leaks for 2 minutes before scanning was begun. After scanning was completed the gas was pumped out for 10 minutes, and the system was ready for the next sample.

Each record was read directly and no corrections for elution behavior were made. This accounts for the minor peaks above mass 32 in the methanol pattern in Table II.

SORPTION IN THE MASS SPECTROMETER

The major limitation to be overcome in this work was sorption on the glass walls of the system. Sorption by stopcock grease was serious only in running the C_9 alcohol. As an aid to pumping out such high boiling compounds, each stopcock was rotated slowly through 360 degrees while the inlet sample volumes were being evacuated. The use of solenoid-actuated mercury valves would eliminate sorption by stopcock grease (*10*).

Water, an alcohol, or an amine in a sample formerly caused trouble, particularly if their presence was unsuspected, because compounds containing OH or NH_2 groups were strongly sorbed on the glass and left backgrounds in the instrument for long periods. Positioning the leak close to the ionization chamber or using an inlet tube heater reduces this difficulty by minimizing the sorption; complex oxygenated mixtures cannot now be analyzed without one of these modifications of the mass spectrometer.

In order to determine the extent of cross contaminations, 3- and 4-carbon atom alcohols were run at inlet pressures close to 65 microns, starting with *n*-propyl alcohol and running methanol

[1] Present address, Explosives Department, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

after each one. As shown in Table I, the methanol was "contaminated" by eluted C_3 and C_4 alcohols to the extent of 0.1 to 0.2%.

The background of these higher alcohols was reduced at least to the equivalent of 0.02% of the 65-micron pressure given above prior to introducing methanol. By focusing the base or 100% peak of each of these alcohols on the collector slit, the peak's behavior could be recorded as it was reduced during evacuation over a 10-minute period and as it was increased again by methanol elution. During the elution period the base peak rose sharply to a maximum, equivalent to several per cent of the methanol pressure, and then fell asymptotically within 3 to 4 minutes to a minimum value. This value fell below the initial background value when the methanol was pumped out of the system.

In the same manner several aliphatic acids were shown to behave similarly with respect to formic or acetic acid. Other examples are hydrogen sulfide, which elutes mercaptans (thiols) and alkyl sulfides (*15*), and ammonia, which elutes primary, secondary, and tertiary amines. In this laboratory it was further observed that aldehydes, esters, and ketones do not elute alcohols to a significant extent. Thus there appears to be an equilibrium between homologs, in which the lower molecular weight member is able to elute the members of the series above it, probably because of the greater concentration of the elutant even though it is less strongly sorbed.

These experiments made it clear that background runs, as commonly made with no sample in the inlet system, might be of little value if sorbed compounds were involved. In such cases background runs preferably should be made using a low boiling member of the class of compounds to be analyzed.

As a corollary of these observations, the mass spectrometer can be purged of large amounts of strongly sorbed substances by repeated flushing with an appropriate compound—for instance, a large water background was frequently purged from the spectrometer using methanol. Occasionally a liquid sample contained 50 to 90% of water. Rather than attempt to dry such samples, they were run directly. With successive methanol purges the water background was reduced within an hour to a value equivalent to 0.3 to 0.5 micron (0.5 to 1%) of pressure in the inlet system.

Table I. Elution of C_3 and C_4 Alcohols by Methanol

Desorbed Alcohol	Contamination of Methanol, Mole %
Propyl	0.1
Isopropyl	0.08
Allyl	0.2
Butyl	0.2
Isobutyl	0.2
sec-Butyl	0.1

EXPERIMENTAL METHOD

A known solution of eight aliphatic alcohols was prepared by weighing and the composition was calculated as mole per cent. The individual alcohols and the eight-component solution were run as a group during each of several days, so that the deviation of the averaged results from the known composition was determined experimentally.

A second known mixture was prepared with water and the first four aliphatic acids, Eastman white label grade and not otherwise purified. Water was included because it was likely to be present in such mixtures. The acid mixture was run four times in succession after the pure acids were run.

An alcohol-ester solution was studied. This routine laboratory sample was originally of unknown composition. It was run once and the mass spectrometer results were compared with the chemical analyses performed upon it.

The nonyl alcohol samples were experimental products which were fractionally distilled so that the mass spectra of the cuts could be compared. Each fraction was run in duplicate to be sure that sorption did not affect the patterns.

The method of resolving mass spectral data is general and involves the solution of linear simultaneous equations (*14*). As detailed alcohol and aliphatic acid spectra have been published in the American Petroleum Institute's Catalog of Mass Spectra, only the necessary portions of the spectra have been assembled as matrix coefficients in Tables II, IV, and VII. The peaks chosen to define each component are listed at the left, so that in Table II propyl alcohol is determined by the 60 peak, *sec*-butyl alcohol is determined by the 59 peak, etc. (*1*).

In the next to the last column of each matrix is given the observed intensity in divisions of galvanometer deflection. The simultaneous equations were solved and the resulting base peak intensities used to compute the intensity of some of the mixture peaks as a check on the computation (it is not necessary to determine them all). The intensities of a few check peaks are also calculated, and are given in the final column. The agreements between the observed and computed values are measures of the consistency of the equations. Finally, the base peak intensities divided by the respective sensitivities of the pure components define the partial pressures of the components in the inlet bottle. Mole per cents are obtained from the partial pressures.

Beneath the computed mole percentage of each component in Tables II, IV, and VII is shown the synthetic mixture composition or the results of chemical analysis.

Table II. Eight-Component Alcohol Matrix with Mixture Data and Results of One Analysis

Mass to Charge Ratio, *m/e*	Pattern Coefficients: Propyl alcohol	*sec*-Butyl alcohol	Allyl alcohol	*n*-Butyl alcohol	Ethyl alcohol	Isopropyl alcohol	Isobutyl alcohol	Methanol	Mixture Peak Intensities: Obsd.	Calcd.
60	0.0816	0.0069	0.0012	0.0008	0.0003	0.0049	0.0031	0.0002	9.4	9.4
59	0.1164	0.2111	0.0136	0.0038	0.0002	0.0379	0.0474	0.0004	27.5	27.5
57	0.0181	0.0354	1.000	0.0741	0.0018	0.0036	0.0423	0.0042	64.0	64.0
56	0.0023	0.0127	0.0881	1.000	0.0012	0.0006	0.0426	0.0021	131.2	131.2
46	0.0009	0.0224	0.0000	0.0057	0.1757	0.0213	0.0014	0.0015	64.1	64.1
45	0.0333	1.000	0.0113	0.0813	0.3938	1.000	0.0527	0.0008	257.1	257.1
43	0.0363	0.1128	0.0667	0.6043	0.0850	0.1748	1.000	0.0036	170.1	170.2
32	0.0221	0.0023	0.0087	0.0148	0.0128	0.0010	0.0131	0.6911	431.1	430.9
Results										
Base peak intensity	101.4	47.7	46.2	122.8	341.6	58.3	42.5	609.7		
Sensitivity, divisions/μ	63.5	58.7	22.5	23.9	32.7	61.8	29.6	18.4		
Partial pressure, μ	1.60	0.81	2.05	5.14	10.45	0.94	1.44	33.14	Σ P.P. = 55.57μ	
Mole %	2.9	1.5	3.7	9.2	18.8	1.7	2.6	59.6		
Mole % synthetic	3.2	1.5	3.6	8.2	18.9	1.7	2.6	60.2		
Check Peak Calculations										
Check peaks										
31	1.000	0.1826	0.5305	0.9072	1.000	0.0564	0.5809	1.000	1233	1225
29	0.1452	0.1383	0.6491	0.2824	0.2355	0.0966	0.2044	0.6308	566.7	565.4
27	0.1559	0.1555	0.3944	0.4642	0.2157	0.1351	0.3835	0.0032	200.1	198.3

Table III. Quadruplicate Analysis of Synthetic Mixture of C_1 to C_4 Alcohols[a]

Alcohol	Run 1	Run 2	Run 3	Run 4	Average	Synthetic	Deviation of Average
Methyl	59.6	59.0	56.1	59.2	58.5	60.2	−1.7
Ethyl	18.8	19.0	21.1	19.7	19.7	18.9	+0.8
Propyl	2.9	2.9	3.6	3.3	3.2	3.2	0
Isopropyl	1.7	1.4	1.7	1.9	1.7	1.7	0
Allyl	3.7	3.5	4.2	3.8	3.8	3.6	+0.2
Butyl	9.2	10.1	9.0	8.3	9.2	8.2	+1.0
Isobutyl	2.6	2.5	2.8	2.6	2.6	2.6	0
sec-Butyl	1.5	1.6	1.6	1.2	1.5	1.5	0

[a] Data obtained 4 times during 2-week period. Results are in mole %.

Table IV. Four-Component Aliphatic Acid Matrix with Mixture Data and Results of One Analysis (Dry Basis)[a]

Mass to Charge Ratio, *m/e*	Pattern Coefficients: Propionic acid	Butyric acid	Acetic acid	Formic acid	Mixture Peak Intensities: Obsd.	Calcd.
57	0.190	0.0044	0	0	21.0	21.0
41	0.0120	0.273	0.0512	0	44.7	44.7
43	0.0317	0.251	1.000	0	74.4	74.4
29	0.857	0.217	0.178	1.00	1548.0	1548.5
Results						
Base peak intensity	106.8	152.9	32.6	1418[b]		
Sensitivity divisions/μ	35.1	50.8	33.7	35.1		
Partial pressure, μ	3.04	3.01	0.97	40.4	Σ partial pressure = 47.4 μ	
Mole %	6.4	6.3	2.0	85.2		
Mole % synthetic	5.8	5.6	2.5	86.1		
Check Peak Calculations						
Check peaks						
45	0.468	0.221	0.896	0.502[c]	740	825
46	0.0449	0.0078	0.0141	0.649[c]	801	927
60	0.0081	1.00	0.512	0	166	170.5
73	0.274	0.301	0	0	72.1	75.3
74	0.456	0.0133	0	0	48.2	50.7

[a] Sample contained 20% H_2O.
[b] This figure would be 1233 if 46 peak were used in place of 29. Mole %'s then become 7.2, 7.2, 2.3, and 83.3, respectively.
[c] Pattern values of 0.442 and 0.560 are required to fit data.

DISCUSSION OF RESULTS

Alcohols. Table II shows an outline of the computation for one analysis and Table III presents the results of four analyses on this mixture during a period of 2 weeks. Considering the sample complexity, the results of Table III are in good agreement.

Acids. In Table IV are shown the data used to resolve the acid mixture on a dry basis. The results were computed on a dry basis, although the sample was made to contain 20 mole % of water. No attempt was made to determine water by means of the mass spectrometer because the Karl Fischer reagent method was preferred (*7*). The results were in sufficiently good agreement to make the method useful, but a curious anomaly was observed. Better agreement with the synthetic values was secured if the 29 peak was used in the matrix to calculate the formic acid concentration. Normally the 46 peak would be selected, and the composition computed using the 46 peak is given at the bottom of Table IV. This effect does not appear to be associated with reproducibility, because consecutively run portions of the mixture behaved normally as shown in Table V.

Because of this behavior, improved results should be expected if, after making an unknown analysis as indicated, a mixture of similar known composition is run, adjusted approximately to the "unknown" results, and rerun to reproduce more closely the spectrum of the unknown. Water should be included according to the Karl Fischer reagent analysis of the unknown. A difference method may then be used to compute the results.

ALCOHOL-ESTER MIXTURE

Tables VI and VII give the data and results for this analysis of a routine unknown sample. Four carbon alcohols and their formate and acetate esters were suggested components. The chemical analyses for combined C_4 alcohol and combined butyl ester as formate were in agreement with the mass spectrometer results. However, the agreement shown on the check peaks was only fair and at least one other component probably was present. It is evident that the *n* and isobutyl acetates are absent or very low in concentration, because the 61 and 73 check peak residuals are small. The subject of check peaks in unknown mixtures is discussed further below.

The butyl alcohol patterns and sensitivities used in this analysis were not the same as those used in the eight-component alcohol problem (Table II). The alcohol-ester analysis was made after conversion to C.E.C. Model 21-102 and the ionization chamber temperature and other operating conditions were different, as described above.

MASS SPECTROMETER AS COMPARISON DEVICE FOR PURITY AND CONTROL

Occasionally materials are encountered for which no reference standards are available or in which some components are unidentified. Provided the components have significantly different spectra, the mass spectrometer may be used to check for purity or

Table V. Reproducibility of Mass Spectrum for Aliphatic Acid Mixture of Table VI

(In % of *m/e* 29)

Mass to Charge Ratio, (*m/e*)	Run 1	Run 2	Run 3	Run 4	Average
27	13.31	13.45	12.89	13.01	13.17
28	25.97	26.56	25.54	25.81	25.97
29	100	100	100	100	100
44	13.92	14.25	12.97	13.64	13.70
45	49.90	50.11	47.70	49.74	49.86
46	50.04	50.11	50.19	50.43	50.19
60	17.52	17.62	16.42	16.52	17.02
73	6.97	6.95	6.67	6.19	6.70
74	3.99	3.92	3.91	3.84	3.91

Table VI. Relative Intensities of Principal Peaks of Some Butyl Esters

(Ionization chamber at 240° C.)

Mass to Charge Ratio, *m/e*	Mass Spectrometer, C.E.C. 21-102: Butyl formate	Isobutyl formate	Butyl acetate	Isobutyl acetate
27	46.5	4.98	12.4	8.52
28	20.7	46.8	5.37	2.35
29	53.3	51.3	12.6	8.00
31	65.4	62.9	2.07	1.60
41	61.1	71.2	14.6	11.3
42	15.8	25.3	3.99	3.51
43	47.8	100	100	100
45	7.56	2.42	0.93	0.91
55	12.3	6.90	5.62	1.92
56	100	77.8	32.3	23.8
57	14.0	8.86	4.28	3.35
60	1.86	38.0	0.26	0.10
61	4.36	10.3	11.1	2.52
73	7.33	1.41	12.5	14.7
74	0.94	1.09	0.46	1.40
85	1.17	....	....	....
102	0.82	0.57	....	....
116	....	....	0.08	0.11
Sensitivity[a]	43.7	35.8	137	154
Sensitivity of *n*-butane, 43 peak	70.1			
n-Butane, 58 peak	11.02% of *n*-butane 43 peak			

[a] Sensitivities calculated as division per micron for 100% peak.

Table VII. Butyl Alcohol and Formate Matrix with Mixture Data and Results of Analysis

Mass to Charge Ratio, m/e	n-Butyl Alcohol[a]	Isobutyl Alcohol[a]	Butyl Formate	Isobutyl Formate	Mixture Peak Intensities Obsd.	Mixture Peak Intensities Calcd.
31	1.00	0.658	0.654	0.629	583	583
74	0.0097	0.0921	0.0094	0.0109	26.2	26.2
29	0.336	0.256	0.533	0.513	231	231
60	0.0008	0.0030	0.0186	0.380	9.5	9.6
Results						
Base peak intensity	370	236	67.2	20.0		
Sensitivity divisions/μ	45.8	50.1	43.7	35.8		
Partial pressure, μ	8.08	4.72	1.54	0.56	Σ partial pressure = 14.90μ	
Mole %	54.3	31.7	10.3	3.8		
Mole % chemical method	88		11.8			
Check Peak Calculations						
Check peaks						
41	0.618	0.586	0.611	0.712	431	422
42	0.322	0.588	0.158	0.253	280	274
43	0.601	1.00	0.478	1.00	543	510
45	0.0747	0.0503	0.0756	0.0242	38.0	45
55	0.121	0.0465	0.123	0.0690	68.6	65.4
56	0.863	0.0385	1.00	0.778	394	411
57	0.0650	0.0405	0.140	0.0886	62.3	44.8
61	0	0	0.0436	0.103	4.2	5.0
73	0.0136	0.0178	0.0733	0.0141	16.2	14.4

[a] Pattern coefficients for mass spectrometer C.E.C. 21-102 are different from corresponding ones for C.E.C. 21-101 (Table III).

Table VIII. Mass Patterns of 3,5,5-Trimethyl-hexan-1-ol

Mass to Charge Ratio, m/e	Du Pont Nonyl Alcohol Refined	Du Pont Nonyl Alcohol Heart cut of refined	Isomeric Nonyl Alcohol Mixture
27	15.1	15.0	30.7
29	24.6	27.2	39.7
31	14.7	17.1	26.2
41	38.5	38.6	66.1
43	23.0	21.5	68.9
55	22.3	21.6	58.4
57	100.0	100.0	100.0
69	26.1	24.7	42.1
70	9.9	9.2	32.6
71	8.4	7.6	35.3
83	6.1	5.4	29.0
87	8.6	8.8	10.1
111	5.1	4.9	5.1
129	4.8	5.0	2.8

conformance to a given mixture specification. For example, no reference standards were available for the analysis of C_9 alcohol mixtures, and yet useful results were obtained. One of the principal components was 3,5,5-trimethylhexan-1-ol, which boils at 194° C. This substance was one of the highest boiling, strongly sorbed organic compounds used to obtain a mass spectrum in this laboratory.

Several samples were examined, but for the purpose of this paper only the comparison of two samples is given. One of these was the Du Pont commercial product which was known to consist essentially of the single nonyl alcohol isomer 3,5,5-trimethylhexan-1-ol (*3*). The other sample was a mixture of nonyl alcohol isomers prepared by a different process.

The two samples as received were run on the mass spectrometer. In addition to this, each sample was subjected to an analytical distillation and the mass spectra of the several cuts were obtained. All the data were computed from the second run of successive introductions of each sample or cut in order to minimize sorption effects.

The results of the sample runs and the refined Du Pont nonyl alcohol heart cut are shown in Table VIII. It is evident that the heart cut and the sample from which it was distilled have very similar mass spectra, whereas the mixture sample spectrum is very different. This is conclusive evidence that the samples are different materials.

The mass spectra of the distillation cuts were taken to determine the extent of pattern variations. It was observed that the ratio of the 41 and 57 peaks represented fully the characteristics of the other pattern values. The Du Pont refined nonyl alcohol pattern was constant within 1 part in 200 until 96.5% of the sample had been distilled. The pattern of the 3.5% residue fraction increased from the heart cut value of 38.6 to 44.8%. With a boiling range at 150 mm. of 141–142° C., these figures indicate a high concentration of a single isomer. This assumes that the mass patterns of the various nonyl alcohol isomers are different, even though some of their boiling points may be close together. The assumption is supported by the isomeric mixture data. This sample boiled over a 20° C. range at 150 mm. The 41/57 ratio of the various cuts ranged from 62% in the foreshots to 35% in the heart cut range and to 160% in the final fraction. The variations between the refined sample and the mixture sample were thus accentuated.

ANALYSIS OF UNKNOWN OXYGENATED MIXTURES

In analyzing unknown mixtures the first requirement is that the components be qualitatively identified. Mass spectrometer data alone are often inadequate for this purpose, as essentially the same group of peaks is obtained for acids, esters, alcohols, and ethers. Their parent masses (molecular weight calculated using atomic weights of most abundant isotopes—e.g., 32 for methanol) are all two units above the parent masses of the paraffin series. As shown in previously published data (*5*, *6*, *12*), it is clear that peaks one and two units above the paraffinic parent masses are most typical. These compounds are therefore easily recognized as a group in mixtures of hydrocarbons. Aldehydes and ketones, on the other hand, have parent masses identical with those of the paraffinic series, starting at 30 for formaldehyde and ethane, at 44 for acetaldehyde and propane, at 58 for acetone, propionaldehyde, and the butanes, and so on. These two classes are therefore not readily recognized in hydrocarbon mixtures containing paraffins. On the other hand, the intensities of the several peaks and the recognizable parent mass values when used with the pattern files and a knowledge of the sample source frequently permit computers to select probable components. Quantitative chemical determinations of many functional groups are readily performed and help to define the composition. More detailed information can obviously be obtained by examining the fractions of an analytical distillation, using chemical methods and both the mass and infrared spectrometers. These combined tools make an analytical combination of great versatility, and they must be used together to appreciate their capabilities.

It is not generally practical to apply such leverage to routine samples, and the mass spectrometer computer usually must base his work on the observed spectra and on his knowledge of the sample sources. As only major constituents are usually of interest in these samples, the computer may expect and tolerate appreciable residual intensities on peaks not used to derive his solution. If the residuals permit an identification, the newly recognized compound can be included in the computations.

In general, the agreement between the observed and calculated values of check peak intensities should be better for known than for unknown mixtures. With the former, each component is used as a calibrating standard, and any impurities simply contribute their spectra as part of the standard. But with an unknown

mixture significant positive or negative residual differences probably mean that one or more components have not been identified. Negative differences on check peaks arise because an unidentified component permits too much of some components to be subtracted from the peaks used to calculate the results.

As molecular weights of sample components increase and vapor pressures decrease, the difficulty of delivering a representative portion of the sample as vapor to the ionization chamber increases. The components of each mixture must be studied alone and in combination in order to assess the instrument's capabilities, including sorption and pump-out behavior. In the author's researches it has been possible with the aid of eluting techniques to pump out all backgrounds so far encountered. These include backgrounds due to C_5 aliphatic acids and C_8 and C_9 alcohols.

Numerous applications of mass spectrometry to plant control may be envisaged. Consider a hypothetical process involving partial oxidation of hydrocarbons to a mixture of liquid oxygenated intermediates with vapor pressures suitable for mass spectrometer scanning. Starting with the crude product stream and continuing with streams from subsequent steps of purification, the mass spectrometer might permit process controls without all the labor of detailed analyses. These streams are often too complex to permit complete analyses to be made in time to be useful in plant operation. Furthermore, plant operation does not ordinarily require a complete analysis. It only requires information to show that the process is in or out of control. The process sample could be compared with two standards: one containing an increased amount of lower boiling components in addition to the standard amounts of the remaining materials and the other containing the standard amounts of low and middle range components and an increased amount of high boilers. Deviation from control in either direction should be recognizable from the mass spectrometer patterns. Appropriate process variables could then be adjusted.

CONCLUSIONS

An electric heater operated at 240° C. on the inlet tube of the Consolidated Engineering Corp. mass spectrometer provides a very simple and effective means of extending the instrument's versatility. The heater is currently used for all samples, including hydrocarbon and permanent gases. The reduction in sorption of compounds containing hydroxyl groups has been sufficient to permit analyses of acceptable accuracy on alcohol mixtures, aliphatic acid mixtures, and alcohol-ester mixtures. It has also permitted the comparison of samples with sorption characteristics as strong as those of a C_9 alcohol. There is thus available a broad field of applicability of the mass spectrometer which is as yet very incompletely explored. Part of this unexplored territory lies in the province of process control in plants making oxygenated compounds boiling as high as 200° C. Many process streams require a rapid means of determining whether the system is in or out of control. Mass spectrometer comparisons may provide such a check in many cases.

The elution of compounds by members of their class is effective in purging the system of background peaks. Methanol has been used successfully in removing water background, left after running 80% water samples, in as little as an hour. Nonyl alcohol after 20 runs was removed by methanol in about 8 hours. Nonyl alcohol is the highest boiling strongly sorbed substance run in these laboratories.

Many mixtures of oxygenated compounds may be analyzed if a qualitative identification of the components can be provided. Together with infrared spectrometry, sample history, analytical distillation, and chemical analysis for functional groups, a very powerful team is available for samples which justify the effort. Trace quantities of impurities in process streams may be concentrated, identified, and analyzed by the combined abilities of the classical and instrumental methods of analysis.

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Determination of Moisture in Gases by Automatic Dew Point Equipment

R. M. ILFELD, *General Engineering Laboratory, General Electric Co., Schenectady, N. Y.*

The limitations of manually operated dew point equipment have stimulated the development of an automatic dew point recorder. The resulting instrument will record the dew point temperature of a gas over the temperature range from ambient to −90° F. The instrument comprises a mechanical refrigerator, a heater-controlled mirror assembly, and electronic controls to observe and control the amount of dew formed. The mirror reflectance is monitored by a photocell, and the resulting signal converted into an increase or decrease in the amount of heat supplied to the mirror. The automatic dew point recorder gives a continuous record of dew point without constant attention, and at the same time eliminates errors due to differences in operators. The measurement is made at an equilibrium dew point temperature rather than the transient temperature often used in manual observation. The equipment is designed for normal service under industrial plant conditions.

THE availability of automatic dew point equipment has made more convenient the application of the dew point method to industrial problems. This paper describes the dew point recorder and indicates some of the advantages of automatic over manual apparatus.

PRINCIPLES OF DEW POINT MEASUREMENT

A characteristic of the dew point method of moisture determination is the fact that dew point measurements give absolute humidity, independent of ambient conditions. Dew point measurement is a temperature measurement. The dew point is the temperature at which the air or test gas becomes saturated with moisture, the temperature at which moisture will begin to condense onto a cooling surface. Care is needed in the interpretation of the observed dew point temperature in terms of actual moisture content. At temperatures above freezing, calculations of moisture content based on vapor pressures over water agree closely with experiment. Below freezing, to temperatures as low as −90° F., theory predicts that data on vapor pressure over ice should be used to calculate moisture content. Experiments show, however, that calculations based on extrapolated vapor pressures over water agree more closely with moisture contents determined by other methods, even though water cannot exist at these temperatures (*1–3, 5*). Table I is the compromise data recommended by General Electric for interpretation of dew point temperatures measured with its dew point recorder.

MANUALLY OPERATED DEW POINT APPARATUS

The dew point indicator shown in Figures 1 and 2 was built for industrial use.

The mirror surface is cooled by a tank of liquid carbon dioxide, which is allowed to expand through an adjustable throttling valve. The cooling gas flow is closely controlled, to provide a sensitive indication of the presence of dew on the mirror surface.

Test gas is introduced into the mirror chamber, where the first surface contacted is the mirror itself. A light and a small viewer are provided to observe the mirror surface, and the temperature is measured by a thermocouple in a potentiometer circuit. The operator adjusts the flow of cooling gas until a faint presence of dew is observed. The temperature is read directly on a temperature indicator as the dew point. The accuracy of this equipment may be as good as 2° or 3° F.

The unit is portable and includes a pressure regulator and adjustable valve for the cooling gas supply.

AUTOMATIC DEW POINT RECORDER

While the manually operated equipment described above is suitable for laboratory and sampling operation, in many industrial processes automatic recording equipment is required. The operation of the dew point recorder is shown in Figure 3.

A two-stage refrigerator, of more or less conventional design, is capable of cooling the mirror assembly to a temperature of −90° F. This makes possible measurement of dew points down to as

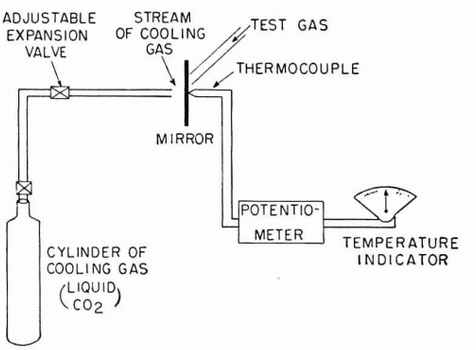

Figure 1. Diagram of Accurate Dew Point Indicator

Including carbon dioxide cooling gas and thermocouple potentiometer. Flow of cooling gas is adjusted until dew spot is seen. Dew point read on temperature indicator

Figure 2. Portable Dew Point Indicator

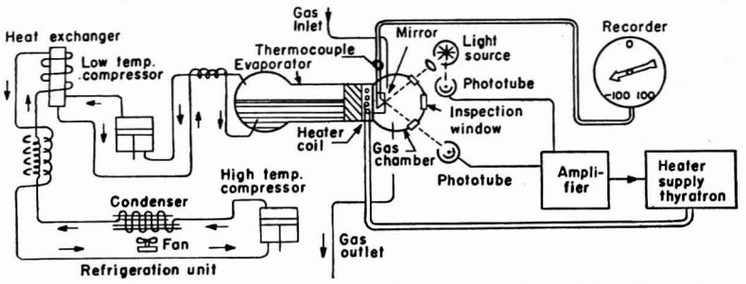

Figure 3. Schematic Diagram of Automatic Dew Point Recorder

low temperatures as are required in normal commercial measurements. Mirror temperature at the dew point is measured and recorded by a conventional thermocouple and electronic recorder combination; the thermocouple is placed as near the surface of the mirror as possible, to give an accurate indication of the mirror surface temperature at the dew point.

The presence of dew on the mirror is observed by a simple photoelectric system. A small light source provides a beam of light which is reflected from the surface of the mirror into a photoelectric tube. The output of this phototube, and of a second phototube which continuously monitors the light intensity from the source, is amplified to provide a control signal for adjusting the temperature of the mirror.

Rapid response of the automatic dew point recorder is obtained with a heater assembly used to control mirror temperature. This heater coil consists of a pancake-type winding located close to the mirror surface, with sufficient heat capacity to raise the mirror temperature in opposition to the refrigerating system. Thus the mirror temperature is continually regulated by an electrical heat supply working in opposition to the constant refrigerator cooling. The electrical power supplied to this heater is controlled by a thyratron unit, which in turn is controlled by the signal from the phototube amplifier. The entire system is adjusted to maintain a specified size of dew spot on the mirror at all times, giving a constant indication of dew point temperature on the recorder.

Table I. Conversion of Dew Point Temperatures to Moisture Content

Amount of water vapor in air or other gas at various dew point temperatures, at a pressure of 1 atmosphere (14.7 pounds per square inch). Values obtained by calculations based on vapor pressures over water.

Dew Point Temp., ° F.	Moisture Content Lb./1000 cu. feet	Mg./liter	% by volume[a]	Dew Point Temp., ° F.	Moisture Content Lb./1000 cu. feet	Mg./liter	% by volume[a]
110	3.77	60.5	8.70	16	0.160	2.56	0.308
108	3.57	57.0	8.20	14	0.147	2.35	0.282
106	3.38	54.0	7.75	12	0.135	2.16	0.258
104	3.20	51.0	7.30	10	0.124	1.99	0.236
102	3.02	48.5	6.90	8	0.114	1.83	0.216
100	2.86	45.6	6.45	6	0.105	1.68	0.198
98	2.70	43.2	6.10	4	0.096	1.54	0.180
96	2.55	40.8	5.75	2	0.088	1.41	0.165
94	2.41	38.7	5.40	0	0.081	1.30	0.150
92	2.27	36.4	5.05	−2	0.074	1.18	0.136
90	2.14	34.3	4.75	−4	0.0671	1.08	0.124
88	2.02	32.4	4.46	−6	0.0612	0.982	0.113
86	1.90	30.5	4.18	−8	0.0558	0.896	0.102
84	1.79	28.7	3.92	−10	0.0508	0.815	0.093
82	1.68	26.9	3.68	−12	0.0462	0.742	0.084
80	1.58	25.3	3.46	−14	0.0420	0.674	0.076
78	1.49	23.9	3.22	−16	0.0381	0.610	0.0685
76	1.40	22.5	3.02	−18	0.0346	0.555	0.0619
74	1.31	21.0	2.84	−20	0.0314	0.505	0.0558
72	1.23	19.7	2.65	−22	0.0284	0.455	0.0503
70	1.15	18.4	2.47	−24	0.0257	0.410	0.0452
68	1.08	17.3	2.31	−26	0.0232	0.372	0.0407
66	1.01	16.2	2.16	−28	0.0209	0.336	0.0364
64	0.95	15.2	2.02	−30	0.0189	0.303	0.0328
62	0.89	14.2	1.88	−32	0.0170	0.272	0.0294
60	0.83	13.3	1.75	−34	0.0153	0.245	0.0264
58	0.777	12.5	1.63	−36	0.0137	0.220	0.0235
56	0.725	11.6	1.51	−38	0.0123	0.197	0.0210
54	0.677	10.9	1.40	−40	0.0110	0.177	0.0188
52	0.632	10.1	1.30	−42	0.0098	0.157	0.0167
50	0.589	9.5	1.21	−44	0.0088	0.141	0.0149
48	0.549	8.81	1.12	−46	0.0079	0.137	0.0132
46	0.511	8.20	1.04	−48	0.0070	0.112	0.0117
44	0.475	7.62	0.966	−50	0.0063	0.101	0.0104
42	0.442	7.08	0.894	−52	0.0056	0.090	0.0092
40	0.410	6.58	0.827	−54	0.0050	0.080	0.0082
38	0.381	6.12	0.765	−56	0.0044	0.071	0.0072
36	0.354	5.68	0.707	−58	0.0039	0.063	0.0063
34	0.328	5.26	0.653	−60	0.0034	0.054	0.0056
32	0.304	4.88	0.602	−65	0.0025	0.040	0.0041
30	0.280	4.50	0.553	−70	0.0018	0.029	0.0029
28	0.259	4.15	0.511	−75	0.0013	0.021	0.0021
26	0.240	3.84	0.472	−80	0.0009	0.014	0.0015
24	0.221	3.55	0.434	−85	0.0007	0.011	0.0010
22	0.204	3.28	0.398	−90	0.0005	0.008	0.0007
20	0.189	3.02	0.367	−95	0.0003	0.005	0.0005
18	0.174	2.79	0.337	−100	0.0002	0.003	0.0003

[a] Vapor pressures in atmospheres at various dew point temperatures can be obtained by dividing the values for % by volume by 100.

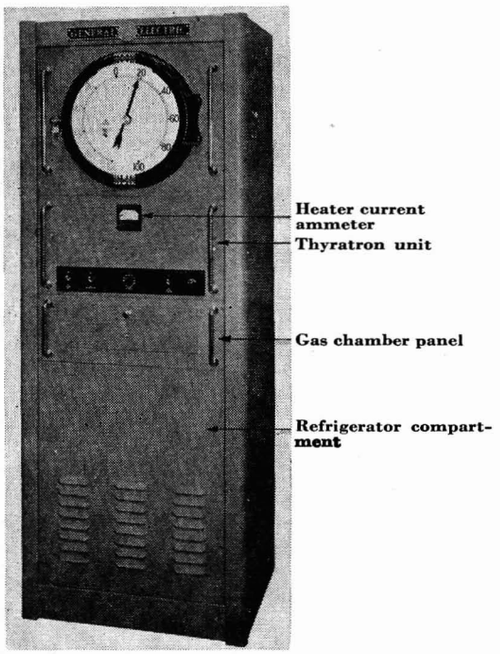

Figure 4. Automatic Dew Point Recorder with Labeled Components

The dew point recorder measures dew point under essentially equilibrium conditions, independent of operator's skill and timing. Measurements with the recorder are usually considered more reliable than measurements made with the indicator.

PRACTICAL FEATURES OF DEW POINT RECORDER

As the apparatus is designed for industrial applications the equipment must be well constructed for plant use. The automatic dew point recorder in its present form is shown in Figure 4. The temperature recorder fills the top panel, the electronic amplifier and heater controls occupy the second panel, and the gas chamber, phototube, and heater mirror assembly take the third panel. The two-stage mechanical refrigerator is contained in the lower half of the apparatus.

For industrial use an equipment should be designed to operate without frequent servicing. This recorder requires service only once a day, to change the recorder charts and to check the adjustment of the electronic circuits. The formation of foreign vapors on the surface of the mirror is inhibited by automatic hourly clearing. Every hour the heater is automatically turned on full, raising the temperature of the mirror to drive off any condensed vapors which might tend to obscure the dew point.

The accuracy of the dew point recorder is approximately ± 2° F. down to dew point temperatures of −20° F. and approxi-

mately ±5° F. from −20° to −90° F. The instrument is insensitive to changes in flow rates over a reasonable range. The temperature recorder is supplied with high and low alarm contacts to provide either an alarm signal or a control signal.

APPLICATIONS OF AUTOMATIC DEW POINT EQUIPMENT

First in importance of the gases, in which moisture can be determined conveniently by the dew point recorder, is process air, air which has been dried for use in a chemical or other industrial process. Large quantities of nitrogen, hydrogen, and oxygen are used also in industrial processes and can be conveniently handled by a dew point recorder. Carbon monoxide, carbon dioxide, and methane may also be used in quantities in processes where moisture content can be a critical process factor. Moisture in inert gases such as argon or neon is of interest when these gases are used for filling electronic tubes.

There are some factors which limit the application of the dew point method of moisture determination, however. None of the standard instruments is designed to withstand corrosive gases, which could dissolve in the layer of moisture on the surface of the mirror to form acids which attack the mirror. Although small quantities of corrosive substances can be tolerated as impurities, moisture in such gases as hydrogen chloride and hydrogen sulfide cannot be determined using the dew point equipment described. Pressure is another limiting factor on the automatic dew point apparatus; at the present time the instrument is not designed for high pressure operation.

The most important limitation is the presence of condensables other than water in the test gas. Heavy hydrocarbons, lubricating oils, ammonia, or other contaminants in the test gas may condense on the mirror and obscure the dew point, causing the automatic dew point observation system to miss the dew point temperature and give a grossly inaccurate reading. Butane and heavier hydrocarbons in natural gas must be held to low concentration if the natural gas is to be monitored for moisture content by the dew point method. Raw ammonia from an improperly adjusted dissociator can cause erroneous dew point readings when moisture determination is attempted in the nitrogen or hydrogen streams.

SPECIFIC USES OF DEW POINT RECORDER

Figure 5 shows the use of the dew point recorder to control the dryness of an air supply.

The air is brought through filters and washers into a chemical drying tower, where moisture is removed. The dry air leaving the tower is further filtered and is ready to be used in the process. The dew point recorder samples a very small amount of the air leaving the drying system. As the drying tower becomes exhausted, it loses efficiency and the moisture content of the air output increases. When the moisture content has increased to the maximum allowable, a contact on the dew point recorder actuates an alarm and a control system. The control system switches the motor-operated 3-way valves to bring the second dryer on stream and puts the first dryer on a regeneration cycle.

Figure 6 shows an installation of the dew point recorder in a refrigerator manufacturing plant. The compressors and evaporators are dried with warm air from which the moisture has been removed chemically. The system is similar to the air-control system described in the previous paragraph, except that an alarm and manual switching are used.

In an aircraft plant a dew point recorder is used to record the amount of moisture in a dissociated ammonia stream. The equipment gives a continuous indication and record of the moisture content.

At one of the water works stations in Philadelphia, water purification is aided by a fine stream of ozone bubbles in the purifying tank. The ozone is produced in an electrical discharge apparatus, using air which has been carefully dried by both mechanical refrigeration and chemical drying action. The dryness of this air is continuously monitored by the dew point recorder; an alarm signal announces the exhaustion of one set of chemical dryers, so that the operators can switch to a new set.

In the General Electric manufacturing plant where nitrogen-

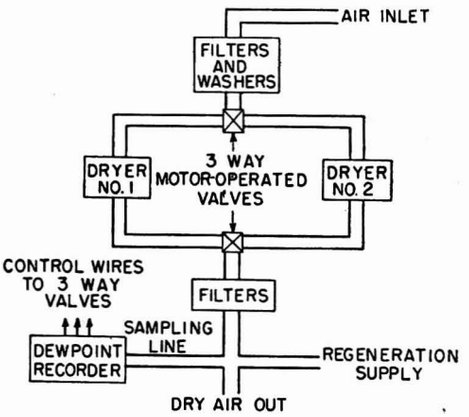

Figure 5. Use of Dew Point Recorder to Control Dryness of Air Supply

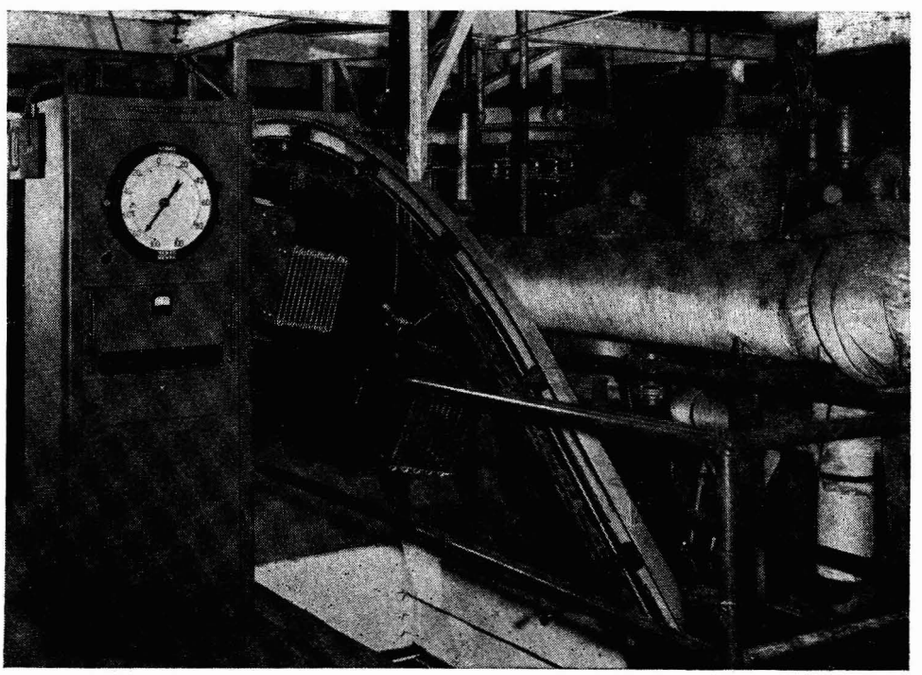

Figure 6. Dew Point Recorder Installed in Refrigerator Manufacturing Plant to Monitor Dryness of Air Used to Dry Refrigerator Components

filled cable is manufactured, the dryness of the nitrogen is monitored by a dew point recorder. Other uses for the equipment have been found in manufacturing operations.

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Automatic Measurement of Optical Rotation

GABOR B. LEVY

Schenley Laboratories, Inc., Lawrenceburg, Ind.

A technique involving an automatic recording polarimeter has been established for studying the kinetics of reactions in which a change in optical activity occurs. It lends itself to various analytical chemical applications. In some instances the presence of a catalyst or enzyme can be detected by the reaction order. The activity or concentration of this agent can be determined by the slope of a "rotogram." The concentration of a reactant or reaction product may be determined simultaneously. Determinations show a high degree of accuracy and precision and surpass many analytical methods in simplicity.

THE measurement of optical rotation affords a valuable analytical method because it is nondestructive. It is of particular potential advantage in kinetic studies, in which analyses should be performed without interference with the reaction under investigation. Although these advantages have been generally recognized, the technique is not employed frequently in research work, because of the tedium of reading values of optical rotation and time simultaneously. Furthermore, only isolated data are obtained and the precision of the readings of continually changing rotation falls significantly short of optimum. To eliminate these objections a continuous recording polarimeter was constructed (*6*), which registers optical rotation within a range of 5° with a precision of about ±0.005°.

The advantage of automatic operation with respect to precision and economy is apparent. In addition, it permits quantitative evaluation of reaction rates even when they change in the course of the reaction, and determination of the exact location of these changes. In manual operation this would be possible only if an infinite number of points were determined. Therefore this automatic operation, for which the author suggests the name "rotography," permits new applications to analytical problems. Some of these applications are discussed below.

When the course of a reaction is followed by a recording polarimeter, a permanent record, "a rotogram," is obtained, which shows optical rotation on the abscissa and time on the ordinate (Figure 1). (The arrangement of scale expansion causes a return to zero after a travel of every 0.5°. Figure 1 represents the inactivation of a solution of sodium benzylpenicillin in 0.2 *M* phosphate buffer of pH 7.0. Concentration is 3.6 mg. per ml.; 15,000 units of penicillinase were added.) The zero or starting point on the ordinate is conveniently made by starting the recorder when the reactants are mixed, but the first pertinent values recorded on the abscissa are obtained only after the mixture has been placed in the instrument and balance is reached. By conducting the reaction over a long period of time, this initial region of uncertain values of rotation may be reduced to an insignificant portion. Thus, essentially the entire course of the reaction is mapped.

The evaluation of the reaction rate from the curvature of the rotogram is an easy task and changes in rate can often be detected by simple inspection. Of particular interest to analytical applications are the zero-order reactions which appear as a straight line on the rotogram. Deviation from the straight line can easily be detected by inspection.

Because catalytic or enzymatic reactions frequently exhibit zero-order reaction rates in some ranges of concentration, the presence of such catalysts can be detected. Furthermore, in these ranges, the reaction rate, and consequently the slope of the rotogram, are proportional to the concentration of the catalysts or enzymes. This offers a convenient method for their quantitative determination.

An abrupt change in reaction order indicates a change in the reaction mechanism. In a closed system, this is usually caused by the disappearance of a reactant. The location of such a point can be determined with great precision by rotography. If the characteristics of the reaction are sufficiently known and if the reaction is specific to a reactant, the concentration of this component can be determined accurately by the change in rotation between the initial value, corresponding to the control, and the value at the "break" in the rotogram. Optically active impurities, inhibitors, subsequent rearrangements, etc., do not interfere, as this second type of rotographic analysis is based on the determination of absolute differences in optical rotation within a certain phase of a complex reaction.

To illustrate the various rotographic techniques, results obtained with the penicillin-penicillinase system are presented.

INACTIVATION OF PENICILLIN

Abraham and Chain have found (*1*) that penicillin is inactivated rapidly at room temperature by the action of penicillinase. The enzymatic degradation is assumed to be due to the hydrolysis of penicillin to penicilloic acid (*2*).

A typical curve representing the reaction is shown in Figure 1. It is immediately apparent that the reaction is of zero order, essentially, throughout its entire course—i.e., the reaction rate is independent of the penicillin concentration.

According to the theory of Michaelis and Menten (*7*, *11*), this is due to the fact that the rate-determining process is the hydrolysis proper rather than the formation of the enzyme-

substrate complex. The existence of zero order rates for this reaction has been reported (*3*), but by rotography it was possible to ascertain this condition over a wider range of penicillin concentration (up to 40 mg. per ml.).

It is generally accepted that the alkaline hydrolysis of penicillin and the enzymatic inactivation are identical reactions, in that they yield penicilloic acid or its salts. However, it was found (Figure 2, representing the inactivation of a sodium benzylpenicillin solution of 1.8 mg. per ml. at pH 11.0) that the alkaline inactivation is a first-order reaction, while the enzymatic hydrolysis is essentially a zero-order reaction. This illustrates the possibility of detecting the presence of an enzyme as compared to a "chemical" agent, both destructive to penicillin.

A peculiarity of the enzymatic inactivation of penicillin is that initially the optical rotation drops rapidly at a steady rate, then there is an abrupt change, and the rotation continues to drop at a much reduced and diminishing rate (Figure 1). The former is assumed to be due to the destruction of penicillin and formation of penicilloic acid, while the latter is considered to be due to the secondary reactions. Three independent facts support this assumption. First, the values of optical rotation indicate that penicilloic acid is the intermediate reaction product (*8*, *10*). Second, there is no residual penicillin found at the end of the first phase of the reaction. [This was proved by separating the enzyme and penicillin immediately after the break in the rotogram occurred. This was done by extraction, substantially as described (*5*). The final aqueous extract consistently showed no microbiological activity.] Third, reactions characteristic of penicilloic acid (*8*, *10*), were obtained. (This was done by adding an equivalent amount of mercuric chloride to the mixture after completion of the initial reaction. This caused a substantial acceleration of the second phase of the reaction, with the optical rotation approaching zero value. Addition of aqueous iodine solution was found to have the same effect.) These findings indicate that the "initial" zero-order reaction represents the hydrolysis of penicillin to penicilloic acid or its salt and that the change in optical activity is proportional to

Table I. Dependence of Reaction Rate on Penicillin Concentration

Relative Enzyme Concentration	Reaction Rate, Degrees/Min.	Slope, Degrees/Min./ Relative Concentration
0.25	0.033	0.132
0.5	0.066	0.132
0.75	0.095	0.126
1.0	0.127	0.127
1.5	0.204	0.136
2.0	0.268	0.143

Table II. Dependence of Change in Optical Rotation on Penicillin Concentration

Sodium Benzylpenicillin		Difference in Rotation		
γ/ml.	Mg./ml.	Per sample, scale divisions	Corrected for enzyme (−17 divisions)	Slope, °/mg./ml.
1603	0.96	171	154	0.786
3206	1.92	316	299	0.763
4809	2.88	468	451	0.767
6412	3.85	621	604	0.769
8015	4.81	770	753	0.767

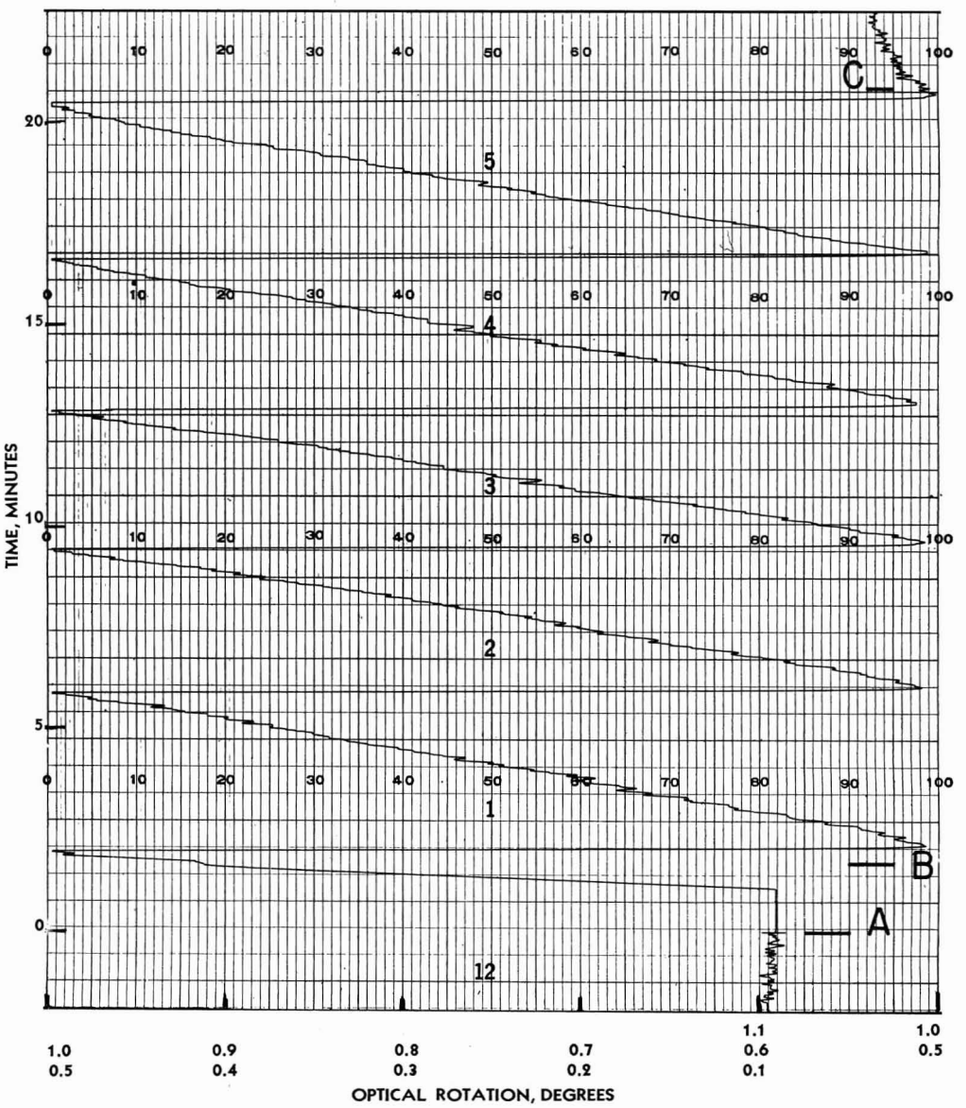

Figure 1. Inactivation of Penicillin

A. Starting point
B. Balance reached
B–C. Main reaction
C. Inflection point

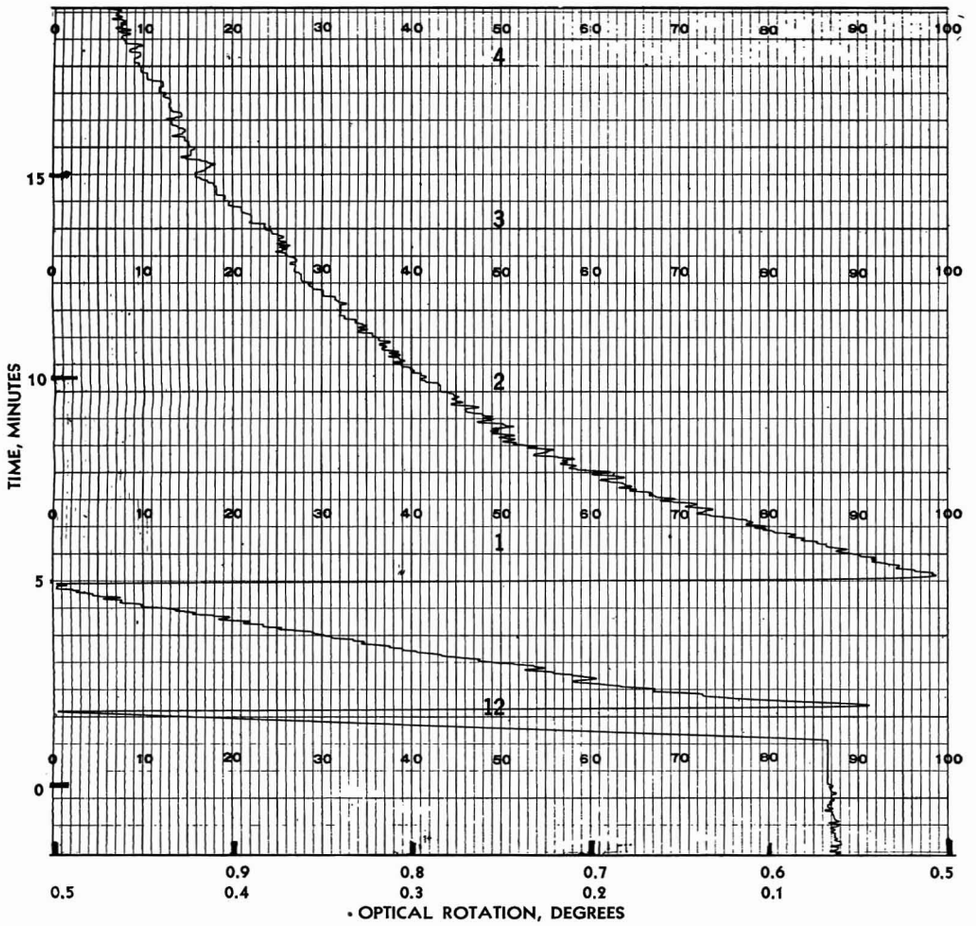

Figure 2. Alkaline Inactivation of Penicillin

Partial rotogram

the penicillin concentration. Consequently, only this phase of the reaction was investigated in detail.

The effect of pH, temperature, and the natureof buffer was studied. It was found that the effect of buffer concentration on the reaction rate is small—viz., about 1% deviation between 0.1 and 4% phosphate concentration—and that two lots of mixed penicillins (G 25%, K 35%, F 40%) were inactivated at a rate significantly lower than pure benzylpenicillin. Details of these studies are omitted; however, they were essential in establishing the analytical methods described below and they form the basis for the new rational unit of penicillinase activity (*4*).

ANALYTICAL APPLICATIONS

Determination of Penicillinase. In the zero-order reaction range, the rate of hydrolysis (the slope of the rotogram) is expected to be directly proportional to the penicillinase concentration. Experimental proof of this is shown in tracings of a set of rotograms (Figure 3). The corresponding values are shown in Table I.

In accordance with the new definition of the penicillinase unit (*4*), a slope of 0.0000089° per minute corresponds to one unit of penicillinase (taking into account the dilution of 50 ml. + 1 ml. and 40-cm. cell). Thus, the values obtained can be incorporated in the calibration curve shown in Figure 4.

A penicillinase preparation is analyzed as follows:

A stock solution of a pure alkali salt of benzylpenicillin is prepared in aqueous 0.2 *M* phosphate buffer of pH 7.0. The range of concentration of this stock solution is preferably between 1 and 5 mg. per ml., but its strength need not be known. To 50 ml. of this solution in a beaker, 1 ml. of the unknown penicillinase solution is added. The solutions are thoroughly mixed, and a 40-cm. polarimeter tube is filled with the mixture. A rotogram is prepared at 25° C. and its slope is measured. For this purpose it suffices to run the rotograph for a few minutes and count the number of divisions for several corresponding values along the abscissa and ordinate. The only precaution necessary is to take the readings of the slope on a straight-line (zero reaction order) section of the rotogram. The penicillinase activity, corresponding to the slope, is read off the calibration curve.

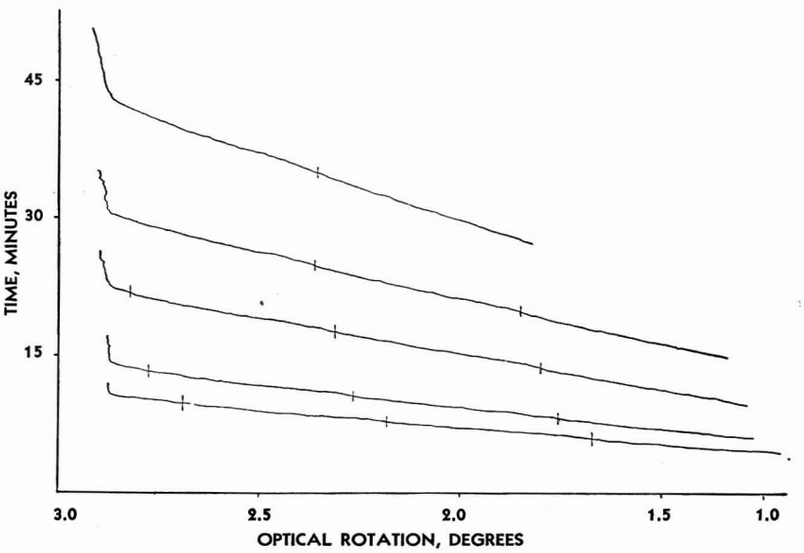

Figure 3. Unfolded Tracings for Enzymatic Inactivation of Penicillin

Vertical lines indicate places where chart sections were joined. Relative enzyme concentration from top curve to bottom curve: 1, 1.5, 2, 3, and 4. Penicillin concentration 4 mg. per ml. in pH 7.0 phosphate buffer

Determination of Penicillin. The "initial" reaction represents the hydrolysis proper of penicillin. At the break or inflection point some penicillin is still present, but this quantity is less than 2 units per ml., as found by assay. It is assumed that the values of rotation at the point of "inflection" do not correspond to the equivalent amount of penicilloates but are somewhat reduced by further degradation. However, the intersection of the two straight lines (corresponding reaction rates) represents a geometrical "end point." The difference in values of rotation between the control—i.e., penicillin solution prior to inactivation—and this end point is a measurable, and as found, a reproducible quantity. The only additional datum necessary to carry out the analysis is the rotation of the penicillinase solution. This cannot be incorporated into the control and therefore its rotation is determined separately.

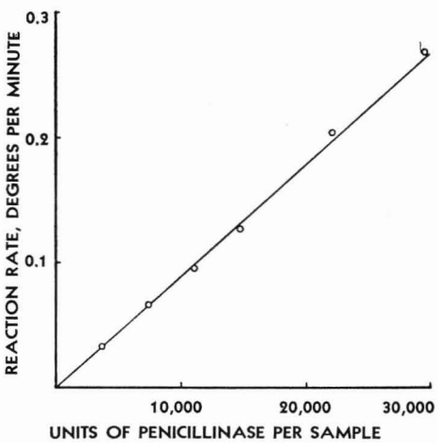

Figure 4. Calibration Curve for Determination of Penicillinase

A penicillin sample is analyzed as follows:

A solution containing the penicillin sample, 0.5 to 5 mg. per ml. with 0.2 *M* phosphate buffer at pH 7.0, is prepared. A portion of this solution is diluted with buffer and is used to balance the instrument. Another portion is diluted in the same proportion by a penicillinase solution (whose strength need not be known) and a complete rotogram of the ensuing reaction is prepared as described above. The rotation of the aliquot of penicillinase solution used in the analysis is determined separately. The change of rotation between the initial value and the value at inflection is determined by counting the divisions along the abscissa in the rotogram. The value found for the penicillinase solution is subtracted. The penicillin concentration corresponding to this value is read off the calibration curve shown in Figure 5 (based on the experimental points shown in Table II).

DISCUSSION

The proposed method for the determination of penicillinase is comparable in precision to other available methods (*4*), and surpasses them in simplicity and ease of operation. The method for the determination of penicillin shows a precision of the order of 1% and is comparable in specificity (accuracy) and precision to the earlier chemical method (*9*) and is complementary to it, in that it permits the analysis of buffered solutions. These analytical methods point to general applicability whenever optically active compounds are involved. Polarimetry and rotography are distinct methods and they are related, to use an analogy, as pH measurement is to potentiometric titration.

By modifying the apparatus and technique, it should be possible to record optical rotation with relation to variables other than time. Some of these modifications are of potential industrial importance. Thus, with volume as the variable, it should be possible to record the quantity of an optically active compound in a varying product stream. When a varying mixture of optically active compounds is involved, the concentration of one could be determined by the use of two instruments linked in such a fashion that the difference of optical rotation is recorded before and after completion of a reaction that is specific to only one component.

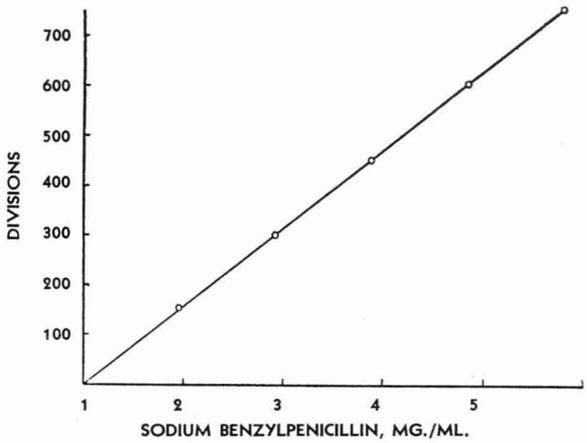

Figure 5. Calibration Curve for Determination of Penicillin

Rotography is defined to include all applications based on the continuous recording of optical rotation with relation to a variable—usually elapsed time. Rotographic analysis is defined as a special case, in which the record or rotogram is used to determine the concentration of a catalyst or enzyme (slope) or concentration of a reactant or reaction product (width).

ACKNOWLEDGMENT

Suggestions of several members of these laboratories are gratefully acknowledged, particularly those of Philip Schwed and David Fergus, as well as the latter's valuable assistance in the experimental work. The author also wishes to thank Kurt Ladenburg and Bruno Puetzer for their interest and encouragement and Schenley Laboratories, Inc., for permission to publish this paper.

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Chromatographic Separation of Phenol from Cresylic Acids

R. J. ZAHNER AND W. B. SWANN

Research and Development Department, Socony-Vacuum Laboratories, Paulsboro, N. J.

Commercial cresylic acids fortified with phenol are used as solvents in some petroleum refinery processes. The phenol content is a critical factor and is adjusted periodically to give optimum results. The methods commonly employed for determination of phenol in cresylic acids are time-consuming and subject to interference by the other components. It was believed, therefore, that rapid separation of phenol from its homologs by partition chromatography would simplify the final estimation, which may be accomplished by a colorimetric or ultraviolet spectrophotometric method. The phenol content may be determined within an accuracy of about 2% of the amount present and the elapsed time for a single analysis is about 2 hours. The operator is able to handle four to six separations at a time. The method has general applicability for the determination of phenol in a wide variety of material, as possible interfering substances are removed in the separation step. Other applications, such as estimation of *o*-, *m*-, and *p*-cresol, are indicated by lengthening the chromatographic column.

CRESYLIC acid is a trade designation of various commercial grades of cresols as distinguished from the sharply fractionated fully refined grades of isomeric cresols. In some grades, the cresols predominate; in others, the predominant constituents are xylenols and the higher boiling homologs of phenol. Cresylic acids recovered from cracked petroleum oils are likely to contain substantial amounts of impurities such as neutral oil, sulfur compounds (mercaptans, thiols), nitrogen bases (pyridine), and thiophenols; whereas the coal tar cresylic acids contain very small amounts, if any, of these materials. Petroleum cresylic acids also contain monohydric phenols with side chains longer than the methyl group. Some of these higher alkyl phenols show very low reactivity and apparently interfere with, or inhibit, the color-forming reactions of the much more reactive phenol, so that the value of colorimetric methods for measuring the phenol content directly in cresylic acid mixtures is questionable. For these reasons the colorimetric method of Chapin (*1*) does not consistently give sufficiently precise results. In addition, the unduly long time (12 hours) required for color development of the phenol complex makes its use in rapid plant control analysis undesirable.

In view of the increasing production and use of cresylic acids in the petroleum industry, available methods for determining the phenol content were reviewed. The method of Miller and Urbain (*6*), wherein a partial purification and subsequent oxidation of the phenol by dichromate are made, appeared to be the most promising one; however, it failed to give repeatable or quantitative results. This conclusion is also substantiated by the work of Warshowsky and Schantz (*8*), who state that "some cresol is also oxidized as well as the phenol." Consequently, a countercurrent distribution method, similar to that described by Warshowsky (*8*), was investigated using a Craig apparatus and technique (*2–5*, *7*, *9*). Although the countercurrent distribution technique was found to be a powerful research tool for making certain difficult separations, it was not adaptable to routine control analysis, owing to the unfavorable time factor of 12 hours by two operators per sample in a 25-plate apparatus.

From the experience gained in a preliminary survey, it became apparent that the same satisfactory results might be obtained as in the countercurrent method if a chromatographic column could be adopted for the separation. This proved to be true, and in conjunction with an ultraviolet absorption technique for estimation of the phenol, the time was decreased to 2 hours per analysis. Furthermore, the simpler chromatographic apparatus enables one operator to run four to six separations simultaneously. Interfering substances are eliminated by the complete separation of the phenol from all other phenolic material present in the sample before the final phenol content is estimated.

THEORY

The underlying principle of partition chromatography is the separation of components of a mixture by partitioning between two immiscible solvents when one of the solvents is held by an adsorptive surface. From solubility studies it was found that the partition coefficient of phenol in the liquid-liquid system cyclohexane-water is appreciably different from the partition coefficient of its homologs.

It was also found that silicic acid with a known amount of water adsorbed on its surface when placed in a typical chromatographic column serves to hold a permanent water layer and permits the passage of a mobile cyclohexane layer over its surface. Such a column then readily lends itself as a tool for the separation of the components of a mixture with respect to their partition coefficients between cyclohexane and water, the component having the greater partition coefficient passing through the column first and the component with the lesser partition coefficient passing through later. After separation, the phenol content of the phenol fraction of the chromatogram may readily be estimated by measuring its absorption of ultraviolet light in the region of 270 mμ. The specific extinction coefficient of phenol in cyclohexane was determined experimentally using known concentrations of purified phenol.

PREPARATION AND STANDARDIZATION OF APPARATUS

The apparatus shown in Figure 1 is used in effecting the separation.

The total length of the column is 250 mm. and the inside diameter is 18 mm. The packing is approximately 150 mm. in length and consists of 19 grams of silicic acid treated with 10 grams of water. The rate of flow of such a column is approximately 130 ml. per hour.

The separated phenol may be estimated by using a Model DU Beckman spectrophotometer or any suitable instrument that will measure ultraviolet absorption in the region of 270 mμ.

Preparation of Column. Grind in a mortar 19 grams of 100-mesh silicic acid (water content, 22% by weight) while slowly adding 10 ml. of water so that the dry powdery appearance is maintained. Add to this about 30 ml. of cyclohexane to form a mobile slurry, and pour it into the column containing a wad of glass wool at its constricted end. Add more cyclohexane to fill the column and apply a pressure of 5 pounds per square inch of nitrogen gas, thus forcing the cyclohexane through the column and

at the same time packing the silicic acid more firmly. Release the pressure when the level of the cyclohexane approaches the surface of the silicic acid. Continue this process until the silicic acid is firmly packed and attains a height of approximately 150 mm. Place a wad of glass wool firmly on the surface of the packing to prevent any disturbance of its surface. Under normal conditions, a column may be used indefinitely.

CAUTION. At no time during operation or storage is it permissible to allow the liquid level to fall below the surface of the packing. If this does happen, the activity of the column will be destroyed, requiring repacking.

The cyclohexane will leach ultraviolet-sensitive material from the packing, if the column remains dormant for more than 2 hours. Such material may be removed by flushing the column with 100 ml. of pure cyclohexane prior to admitting the sample to be analyzed. The pure cyclohexane is percolated by applying pressure in a manner analogous to that of processing a sample.

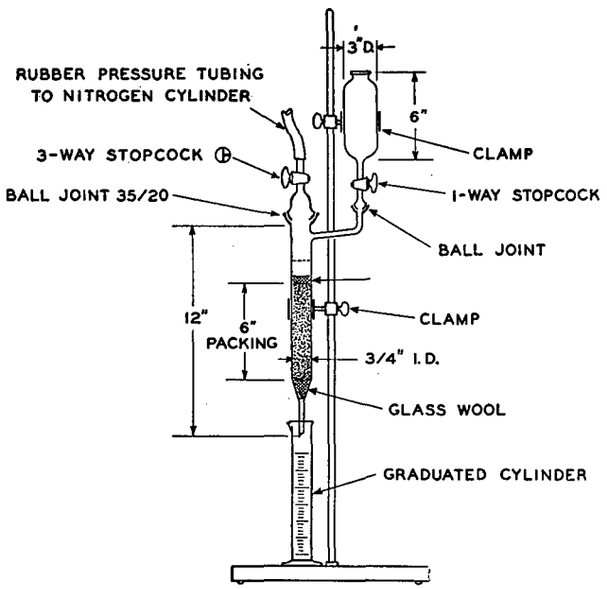

Figure 1. Chromatographic Column

Standardization of Column. A plot of volumes of percolate *vs.* corresponding optical densities exhibits a characteristic minimum point as shown in Figure 2, formed by the fraction which contains essentially no phenolic material. The fractions prior to the minimum point will contain all the homologs of phenol, and those following will contain only phenol. The value of this minimum point is ascertained by standardizing the column with a 1 to 1 mixture of phenol and *m*-cresol. This mixture is used because the partition coefficient of *m*-cresol in a cyclohexane-water system is closest to that of phenol, and hence is the most difficult to separate.

To standardize a column, dissolve a quantity of phenol and *m*-cresol in a volume of cyclohexane in such a manner that 5 ml. of the solution will contain approximately 2 mg. each of phenol and *m*-cresol. Pass a 5-ml. aliquot of the solution into the column and separate by percolating with a 200-ml. volume of cyclohexane. Collect the following fractions of percolate:

Fraction No.	Volume Sequence	Vol. of Fractions, Ml.
1	0 to 50	50
2 to 11	50 to 55; 55 to 60 .... 145 to 150	5 each
12	150 to 200	50
13	200 to 205	5

Make optical density measurements on each of the fractions, using fraction 13 as the blank. Plot the optical density measurements on a graph (Figure 2) as the ordinate and the milliliters of percolate as the abscissa. A stepwise curve will be produced having a minimum which will not vary more than a few milliliters, providing the operating conditions and especially the temperature remain relatively constant.

The standardization is dependent upon the fact that all the phenolic material has been eluded from the column prior to collecting fraction 13. This condition should be checked by measuring the optical density of fraction 13 against a portion of equilibrated cyclohexane that has not been passed through the column; their optical densities should be essentially the same.

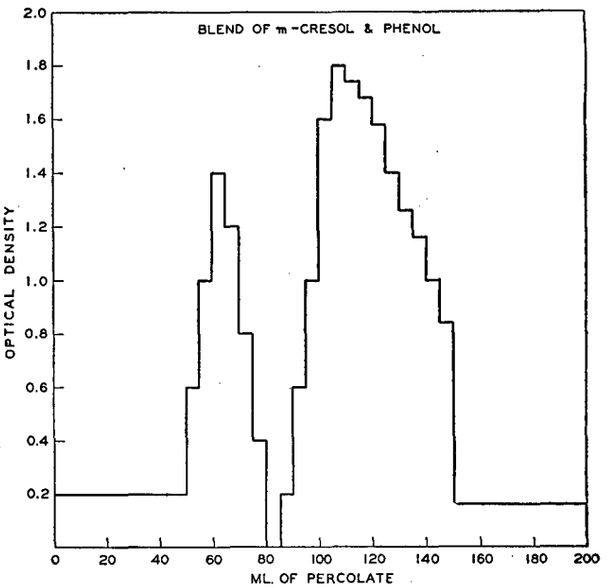

Figure 2. Standardization Curve

If fraction 13 exhibits an optical density greater than the fresh solvent, the eluting process has not been carried far enough.

ANALYTICAL PROCEDURE

Dissolve a quantity of sample in a volume of cyclohexane so that 5 ml. of solution will contain approximately 2 mg. of phenol. Disconnect the ball joint and admit a 5-ml. aliquot of sample solution onto the upper portion of the column, previously prepared as described above. Replace the ball joint and apply 5 pounds of pressure until the cyclohexane just passes into the column packing; at the same time collect the cyclohexane that emerges from the outlet tube in a graduated cylinder. Release the pressure in the column and fill the upper portion with cyclohexane from the reservoir. Again apply pressure to the column, forcing the cyclohexane down until the solvent meniscus approaches the packing surface. Release the pressure and repeat the above operations until the desired amount of cyclohexane has percolated through the column.

Recover the percolate emerging from the outlet tube in stoppered graduated cylinders, labeling each fraction as indicated in the following table:

Fraction No.	Total Vol. of Percolate, Ml.	Vol. of Fraction, Ml.
1	0–70	70
2	70–75	5
3	75–80	5
4	80–85	5
5	85–90	5
6	90–95	5
7	95–195	100
8	195–200	5

The manner in which the fractions are collected is dependent upon the column characteristics. The table was prepared assuming a minimum point between 80 and 85 ml. of percolate.

Discard fraction 1 which contains only the homologs of phenol and make optical density measurements on fractions 2 to 7, using fraction 8 as a blank. Record and plot as in Figure 3 the volumes and optical density measurements obtained for each fraction as performed in the standardization procedure.

CALCULATION

Calculate the total amount of phenol present, using the fraction having the minimum optical density value and all succeeding fractions. Assuming in the above example that fraction 4

had the minimum optical density value, calculate the total amount of phenol present by using the following equation:

$$\% \text{ phenol} = \frac{100 \sum \frac{Av}{e}}{M}$$

where A = absorbance (optical density), v = volume of fraction in milliliters, e = specific extinction coefficient for phenol in cyclohexane, and M = weight in milligrams of original sample in aliquot.

RESULTS

To illustrate the precision of the method, data covering the analysis of a series of samples of known phenol content are presented in Table I. These data indicate a precision of approximately 2%.

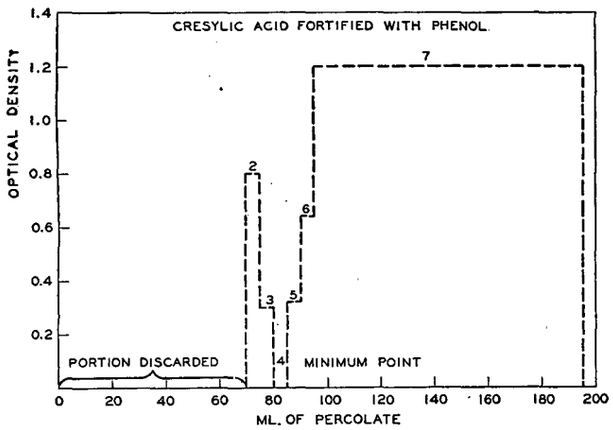

Figure 3. Typical Sample Curve

Table I. Comparative Results Obtained on Known Samples of Cresylic Acid by Partition Chromatography

Sample	Phenol, % Wt. Known	Found
Purified phenol	100	99
Phenol–*m*-cresol blend	55	54
	83	83
Petroleum cresylic acid	10	11
Known blend A	42	40
B	50	52
C	59	60
D	67	68
E	83	81

RESOLUTION OF HIGHER HOMOLOGS

Although the present work was not concerned with the determination of other phenolic compounds, the inherent possibilities of the method may be shown by the graphical chromatogram in Figure 4. This resolution was produced by the passage of a cresylic acid sample through an 8-inch column. The components of the mixture were separated into four distinct bands:

Band	Percolate, Ml.	Component
1	45– 65	Higher boiling phenolic materials
2	65– 80	*o*-Cresol
3	80–135	*m*- and *p*-cresol
4	165–245	Phenol

The column was prepared in a manner analogous to that of the 6-inch column, except that it was packed tighter by tamping with a glass rod. The packing consisted of 27.3 grams of silicic acid and 15 ml. of water.

CONCLUSION

This method provides two essential techniques required in the quantitative analysis of phenol in cresylic acid: a rapid and convenient means of quantitatively separating phenol from its homologs, and a precise and accurate means of determining the quantity of phenol that has been separated.

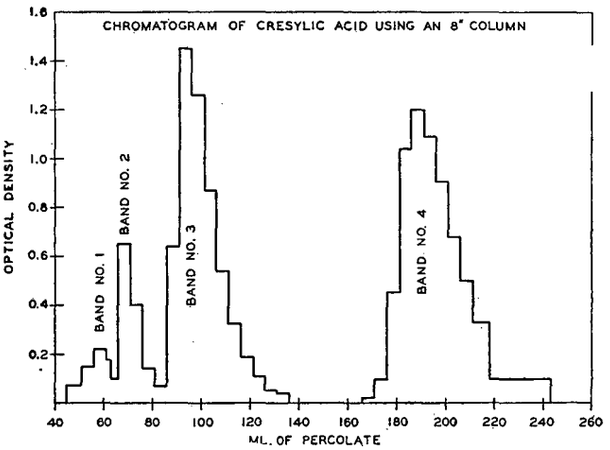

Figure 4. Chromatogram of Cresylic Acid

The use of a spectrophotometer for the final phenol estimation is recommended because of the convenience and accuracy afforded. If an instrument of this type is not available, one of the current colorimetric methods for determining phenol may be employed, as there will be no interfering substances present.

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Techniques and Reactions for Paper Chromatography—Correction

In the article on "Techniques and Reactions for Paper Chromatography" [ANAL. CHEM., **23**, 823 (1951)] the second line of the first column should read "the use of formeous hydrochloric acid," instead of "the use of hydrochloric acid." In the thirteenth line of the second column the address should read Yonkers 5, N. Y. On page 824, fourth line from bottom of second column, "chloroplatinic acid" should have been used instead of "platinochloric acid."

GERRIT TOENNIES

Isolation and Determination of Cobalt

As Nitroso R Salt Complex by Chromatographic Ion Exchange

JOHN A. DEAN, *University of Tennessee, Knoxville, Tenn.*

The sulfonated portion of certain organic reagents was found to enter readily into anion exchange reactions on a column of acid-washed alumina without impairing the ability of the chelate groups to form complexes with metals. Only the surfaces of the alumina particles are covered, rather than having an entire column packed with the organic reagent. With nitroso R salt the color of the cobalt complex is developed in the usual manner, then the entire solution is passed through the alumina column. The cobalt complex and unreacted reagent are removed. Successive elution, first of the excess reagent, and then of the cobalt complex, enables the cobalt complex to be isolated and determined colorimetrically. Combination of the ion exchange technique with the usual colorimetric procedure renders nitroso R salt a specific reagent for cobalt, and the usual interference from copper, chromium, nickel, and iron is eliminated. Twenty minutes' time is required to carry out the chromatographic separation per sample. The method has been successfully applied to all varieties of steels and cast irons, and copper- and nickel-base alloys without prior separations.

ON THE basis of the experience gained while working with sulfonphthalein dyes in an acid-washed alumina column (*1*), it appeared feasible to extend the work to sulfonated organic reagents and to ascertain whether the specificity of certain procedures might be improved. Nitroso R salt was the first to be tried.

The sulfonic acid groups attached to the molecule are readily adsorbed on a perchloric acid–washed alumina column without disturbing the ability of the α-nitroso-β-hydroxyl groups to form an inner complex salt with cobalt. The brilliant red color of the cobalt complex is developed in the ususal manner (*4*); then, following the acidification step, the solution is passed through the alumina column, during which operation only the cobalt complex and excess unreacted nitroso R salt are adsorbed. Successive elution, first of the excess reagent with hot 1 *M* nitric acid, and then of the colored complex with 1 *M* sulfuric acid, enables the cobalt complex to be isolated and its transmittancy subsequently measured. The entire procedure requires approximately 20 minutes following dissolution of the sample.

Compared with previous methods for small amounts of cobalt, the chromatographic ion exchange modification renders the nitroso R salt a specific reagent for cobalt and eliminates the necessity for the removal of copper, chromium, and appreciable quantities of iron, as well as considering any interference from nickel. The method has been successfully applied to a variety of steels, including stainless steels, and copper- and nickel-base alloys without any prior separations.

APPARATUS AND REAGENTS

Apparatus. A Klett-Summerson photoelectric colorimeter, glass cell model, was used for all colorimetric measurements.

A Beckman pH meter, model G, was used for adjustment of pH.

The exchange-adsorption column was prepared in a silver-reductor column obtainable from the G. F. Smith Chemical Co. Several glass beads are placed at the base of the column. Above these are placed 3 cm. of nonadsorbent cotton and then a water slurry of alumina is added until the packed solids extend to a depth of 5 to 6 cm.

Reagents. A standard solution of cobalt, 1.00 ml. = 1.00 mg., was prepared by dissolving 0.263 gram of cobaltous sulfate, dried at 500° C., in distilled water, adding 8 ml. of 72% (12 *N*) perchloric acid, and diluting to 1 liter. A weaker standard solution of cobalt, 1.00 ml. = 0.0100 mg., was prepared by pipetting out 10 ml. of the above solution, adding 7 ml. of 72% perchloric acid, and diluting to 1 liter.

Ammonium acetate or sodium acetate solution, 4 *N*, was prepared by dissolving 308 or 328 grams, respectively, of the C.P. salt in distilled water and diluting to 1 liter.

Nitroso R salt, 1% aqueous solution, was prepared by dissolving 10 grams of the commercial grade salt in 200 ml. of warm water. If a golden yellow solution is not obtained, the solution is passed through a perchloric acid–washed alumina column at the rate of 2 to 3 ml. per minute to remove the dark green ferrous complex so often present as an impurity. This is discontinued when the effluent shows a greenish tint and the column is recharged as described below. The chromatographed liquid is diluted fivefold.

Fisher or Alcoa adsorption alumina was used. The material was repeatedly washed with water and the finely divided portions were decanted and discarded until only a rapidly settling product was obtained.

PROCEDURE

For cast irons and plain carbon steels, about 0.5 gram of sample is dissolved in 5 ml. of 12 *N* perchloric acid plus 10 ml. of distilled water. When dissolution is complete, 1 ml. of 15 *M* nitric acid is added and the solution is boiled several minutes to oxidize all iron salts. Stainless steels should be treated with the minimum quantity of 12 *M* hydrochloric acid required to bring about complete dissolution. Any free carbon is filtered off, the filter is washed well with hot 1% perchloric acid solution, and the filtrate is transferred to a suitable volumetric flask.

For nonferrous alloys the sample is dissolved with 12 ml. of 5 *M* nitric acid. If large quantities of chloride or nitrate ions are introduced during the dissolution of any type of sample, the entire solution should be evaporated to fumes of perchloric acid after any carbon has been filtered off; 5 ml. of 12 *M* perchloric acid are added for this purpose. After the fuming operation, the solution is diluted with 20 ml. of distilled water, 5 ml. of 3% hydrogen peroxide solution are added, and the solution is boiled several minutes to reduce any dichromate or permanganate ions which may have been formed and to expel excess peroxide before the solution is transferred to a volumetric flask.

To the entire sample, or to a suitable aliquot portion, are added 5 ml. of 1% nitroso R salt solution and sufficient 4 *N* ammonium acetate solution to adjust the pH between 5.0 and 5.5. The solution is heated to boiling, then 0.5 ml. of 12 *M* perchloric acid is added for each milliliter of 4 *N* ammonium acetate used. The solution is swirled to dissolve any hydrated oxide precipitate, and allowed to cool to room temperature. Sufficient perchloric acid must be added to ensure the presence of all acetate ions in the form of nonionized acetic acid and to destroy the nitroso R salt complexes of other metal ions.

The sample is passed through the perchloric acid–washed alumina column at the rate of 15 to 20 ml. per minute, rapidly enough so that the individual drops of effluent can barely be discerned. The cobalt nitroso R salt complex forms a brilliant red band at the top of the alumina column, while immediately below will be a diffuse yellow band formed from the excess nitroso R salt. The column is washed with hot (70° to 80° C.) 1 *M* nitric acid solution (free from nitrite ions) until the effluent is colorless when a 25-ml. portion is observed against a white background. This operation elutes the excess nitroso R salt. It may also cause a slight bleeding, up to several millimeters, of the red cobalt band. Too rapid a passage, too hot a solution, or the presence of nitrite ions will cause severe bleeding and possible

elution of some of the cobalt complex. Usually 200 ml. of wash solution are sufficient.

Finally the cobalt complex is eluted with 1 *M* sulfuric acid solution, passed through the column at a rate of 5 to 10 ml. per minute until the effluent appears colorless, then an additional 10-ml. portion of acid is passed. Usually 50 ml. will be required. The eluant is transferred to a 100-ml. volumetric flask and diluted to the mark. The transmittancy is determined at a wave length of 500 to 520 mμ (*5*). Concentrations are determined from a calibration curve. The standard calibration curve is prepared from known amounts of cobalt and reagent which have been similarly developed but not necessarily chromatographed. In the latter event the transmittancy of all standards is corrected for the color adsorption of the excess nitroso R salt reagent. The cobalt complex is stable for 48 hours in the absence of excess dye.

Preparation of Acid-Alumina Column. The alumina column is treated with a 1 *M* solution of perchloric acid until the effluent liquid shows a distinct acid reaction, then washed with several portions of distilled water to remove excess acid.

To recharge the column, it is treated with a 1 *M* solution of sodium hydroxide until the effluent liquid is distinctly basic, washed with several portions of distilled water, and then treated with perchloric acid as described above. Any yellowish material which may be eluted by the sodium hydroxide solution during the recharging operation is ignored. Upon acidification this material turns practically colorless. It will not form a complex with cobalt, nor is it any cobalt complex which has escaped elution with sulfuric acid. Apparently it is either an impurity in the original dye or a decomposition product from the oxidation of the cobalt during the formation of the cobalt complex.

DISCUSSION

The position of an organic sulfonate anion apparently lies intermediate between the nitrate and perchlorate ions in the order for anion exchange adsorption on an acid alumina column (Table I), whereas its position is shifted immediately above that for the nitrate ion when several sulfonate anions are part of the molecule forming a complex salt. Evidently it is more probable that at least one of the six possible sulfonate groups will remain adsorbed at any instant in the presence of nitrate ions, although with only two groups on the dye itself, the dye is easily eluted. Only a slight amount of bleeding (or migration down the column) of the adsorbed cobalt complex anions can be observed during the washing with hot nitric acid unless a solution stronger than 1 *M* is used.

It may seem odd to prepare a perchloric acid–washed column when a nitric acid wash is to be used, as the nitrate ions displace perchlorate ions anyhow. However, a very narrow cobalt band is obtained only if it forms in a perchlorate medium. Subsequent treatment with nitrate ions causes only slight bleeding, whereas if the band is formed in the presence of nitrate ions, excessive bleeding occurs due to the slight difference in exchange ability of the two ions. For a similar reason, sulfate ions are a better eluting agent for removing the cobalt complex than chloride ions, which lie only slightly above the sulfonate ions in exchange ability. The hydroxide ion would be best, except that solutions of it invariably contain traces of ferrous iron and the final eluant would require acidification. The hydroxide ion is so strong an eluant that neutral salt solutions and even an acetic–acetate buffer mixture will cause elution of the cobalt complex although the concentration of hydroxyl ion is extremely low.

It is believed that the reason for the previous serious interference of copper ions has been found. The copper ions form a nitroso R salt complex which is more stable than the corresponding cobalt complex in an acetic acid–acetate buffer. Unless a slight excess of the dye is present—an amount greater than required to form a 1 to 2 mole ratio of copper to dye—no cobalt complex will form in the presence of copper ions. This effect should have been suspected before, as some directions specify extra dye to overcome the interference from copper. The calculated quantity of copper which will react stoichiometrically with 5 ml. of 1% nitroso R salt solution is 4.7 mg., and this has been verified experimentally. Use of extra amounts of reagent, as much as 25 ml. for some copper-base alloys, overcame the interference due to copper ions. Ferrous iron also forms a more stable complex with the dye than cobalt; therefore it is imperative that all iron salts be oxidized during or after the dissolution of the sample.

Chromium(III) proved a more serious interference in the usual colorimetric method than has hitherto been reported. With nitroso R salt it forms an intensely colored complex, identical in color with the cobalt complex, yet fortunately it is not adsorbed on the alumina column, which indicates that a cationic or neutral complex of chromium with the dye is formed.

Nickel interferes, as do several other constituents of the usual alloys and steels in which cobalt is found, in the usual colorimetric procedure by virtue of their colored aquo complexes. Complexation with phosphate has been suggested for the elimination of the interference due to iron (*2*). However, the chromatographic ion exchange modification offers an easy method for the removal of any nonanionic colored substances. Table II shows the results obtained for cobalt by the ion exchange modification on a great variety of samples and in the presence of large amounts of substances hithertofore considered to be serious interferences. The analysis time has been drastically shortened through the elimination of prior separations.

Although the different columns of alumina will vary considerably in adsorptive capacity and degree of packing, the average capacity of a column is 0.05 me. of complex (based on the sulfonate groups available for exchange) or 3.0 mg. of cobalt per cubic centimeter of acid-washed alumina.

Table I. Extended Order for Adsorption on Acid Alumina Column (*3*)

OH^-	$\rightarrow (SO_3^{--}, CrO_4^{--})$	$\rightarrow I^-$	$\rightarrow MnO_4^-$
PO_4^{---}	SO_4^{--}	Br^-	ClO_4^-
$C_2O_4^{--}$	$Cr_2O_7^{--}$	Cl^-	CH_3COO^-
F^-	NO_2^-	NO_3^-	S^{--}

(Ions will be displaced by those preceding them in the series.)

Table II. Analysis of Bureau of Standards Samples by Chromatographic Ion Exchange Method

Sample	Certified Co Value, %	Value Found, %
Ingot iron 55b	0.006	0.005, 0.005, 0.005 0.005, 0.005
36 Ni steel 126a	0.30	0.31, 0.30, 0.31 0.30, 0.30, 0.30
Cast iron 115 (16 Ni–2 Cr–6 Cu)	0.080	0.087, 0.082 0.078, 0.081 0.083, 0.084, 0.082 0.083, 0.087, 0.088
Casting alloy 161 (64 Ni-17 Cr-15 Fe)	0.47[a]	0.49, 0.49, 0.49 0.49, 0.49, 0.50
Cr-Ni(18–9) steel 101c	0.084	0.093, 0.092, 0.095 0.092, 0.091, 0.093 0.087, 0.089
Cr-Ni(18–10) Ti steel 121a	0.090	0.098, 0.096 0.099, 0.099 0.097, 0.096
Cu–Ni–Zn alloy 157	0.136	0.143, 0.140, 0.140 0.142, 0.140, 0.147
66 Ni–29 Cu alloy 162	0.54	0.55, 0.55, 0.54 0.55, 0.54, 0.55

[a] Provisional value.

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Sensitized Paper for Estimation of Mercury Vapor

FRED STITT AND YOSHIO TOMIMATSU
Western Regional Research Laboratory, Albany, Calif.

A sensitized paper was required for use with a portable instrument for estimation of ethylene in air. A method has been developed for preparing sensitized paper, which is both uniform and reproducible (±5%) in its response to mercury vapor. Filter paper is impregnated with red selenium by soaking in potassium selenocyanate solution, draining, and exposing to a hydrogen chloride atmosphere. The quantity of reactive material per unit area of paper is easily controlled by adjusting the concentration of selenocyanate. The properties of this paper have been compared with those of selenium sulfide papers prepared by three different procedures, when used in the form of a strip over which a fixed volume of sample is slowly passed. A temperature of 65° C. or above was required for maximum reactivity of the mercury vapor with the reactive material on each of the sensitized papers. The length of blackening of selenium paper is directly proportional to the mercury vapor concentration and is insensitive to paper temperature between 65° and 200° C. Both selenium and selenium sulfide papers retained their original calibration after a year of storage at room temperature in the dark.

IN THE course of developing a portable instrument for measuring small amounts of ethylene in air by use of hot mercuric oxide as an oxidizing agent (*5*), sensitized paper suitable for detecting and estimating mercury vapor was studied. The use of selenium sulfide for coating paper for this purpose was first described by Nordlander (*4*), who prepared a selenium sulfide powder by a carefully controlled precipitation and drying procedure, followed by a mechanical application of the powder to the surface of the paper. Paper prepared by this method is used in a commercially available mercury vapor detector, in which the quantity of mercury present as vapor is estimated from the degree of darkening which occurs when the reactive surface of the coated paper is exposed to the air in a specified manner. Beckman, McCullough, and Crane describe an improved procedure for preparing and using paper containing selenium sulfide (*1*, *2*). Filter paper is soaked in selenious acid, exposed to hydrogen sulfide, allowed to dry, and then subjected to a baking process. The length of blackening which occurs when a standard volume of sample is slowly passed over a strip of the hot sensitized paper is a measure of the mercury vapor present in the sample.

The authors wished to eliminate the need for separately calibrating each sheet of paper prepared by the method of Beckman, McCullough, and Crane. Omission of the baking step in their procedure produced a uniform, stable, reactive paper with properties that were readily reproducible but rather sensitive to the temperature at which the paper was used. Satisfactory paper with properties relatively insensitive to temperature has been prepared by a method which impregnates the paper with only selenium as the reactive material. The properties of sensitized paper prepared by these methods are compared and the relative advantages of each are discussed.

PREPARATION OF SELENIUM SULFIDE PAPERS

The steps in the preparation of selenium sulfide papers as described by Beckman, McCullough, and Crane (*1*, *2*) were examined. It was verified that the reaction of selenious acid with hydrogen sulfide is quantitative. The time of soaking the paper in the selenious acid may be reduced to 1 to 2 minutes. After soaking, the papers should be drained approximately 15 minutes, but should not be allowed to become dry. Use of a large covered glass jar for the drainage operation eliminates atmospheric relative humidity as a factor; drainage periods from 10 to 60 minutes have yielded satisfactorily uniform sheets under these conditions. If the paper is permitted to air-dry at this stage, it can be humidified at a later date and the preparation completed, but this is not recommended because of the difficulty of uniformly moistening the dried paper. The time of exposure to hydrogen sulfide is not critical so long as reaction is complete; at least twice as long as is required for no further visible change in color is recommended. After these papers have dried, they may be cut into strips and used without further processing. Paper prepared in this manner is referred to below as unbaked selenium sulfide paper.

Within a few days of preparation sheets of unbaked selenium sulfide paper develop an irregular spotty appearance, the spots being of darker hue than the remainder of the paper. After several more days the area of these spots has spread, so that the entire sheet once more appears homogeneous, but of darker color than the original. The colors involved vary from light yellow to deep orange, depending on the concentration of selenious acid used in making the paper. This change in color probably reflects a change in phase of the deposit on the paper, but the exact nature of this change is not understood. Indeed, the nature of the reaction product usually called selenium sulfide is not understood, for available data on the selenium-sulfur phase diagram show no indication of a compound of formula SeS_2 (*3*).

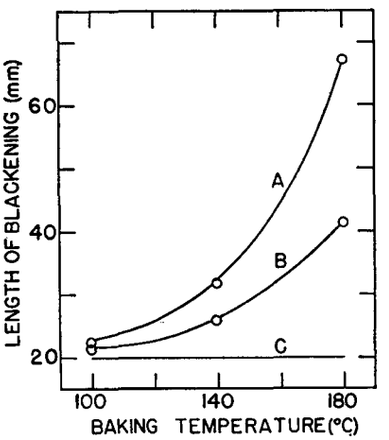

Figure 1. Effect of Baking Conditions on Response of Baked Selenium Sulfide Paper (W-1, 0.10 *M*)

A. **Baking time 30 minutes**
B. **Baking time 10 minutes**
C. **No baking**

Beckman, McCullough, and Crane (*1*, *2*) bake the selenium sulfide paper prepared as above for 30 minutes at 140° C. This baking process is rather critical, because the uniformity and calibration properties of the baked paper depend on how this step is done. The net effect of the process is to remove much of the sulfur and some of the selenium. The effect of varying the time and temperature of baking sheets of selenium sulfide paper on the length of blackening produced by a standard sample of mercury vapor passed over a 0.125-inch wide strip of the baked paper at 150° C. is shown in Figure 1. The horizontal line, *C*,

represents the response when the baking step was omitted. The papers were baked in a forced-draft oven, each sheet being held between two larger sheets of untreated filter paper during the baking process. Increase of temperature or baking time is seen to result in increased sublimation, as indicated by the longer length of strip required to react with the mercury vapor. When sheets were baked between glass plates or in sealed tubes, sublimation was prevented and these papers showed the same calibration properties as unbaked paper.

PREPARATION OF SELENIUM PAPERS

The difficulty of preparing baked selenium sulfide papers with reproducible calibration properties, and the sensitivity of response of unbaked selenium sulfide papers to the temperature at which they are used (see below), indicated the need for a method of preparing paper with calibration properties which are easily reproduced and are relatively insensitive to temperature. Because the temperature sensitivity of response of unbaked selenium sulfide papers above 65° C. was shown to be due to sublimation of active material from the paper, preparation of a paper containing selenium but no sulfur as the active agent was undertaken.

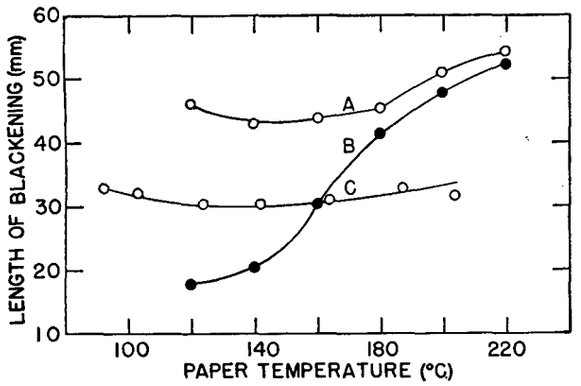

Figure 2. Effect of Temperature on Response of Sensitized Paper

A, B. **Baked and unbaked selenium sulfide paper (W-1, 0.10 *M*), sample volume 72 cc.**
C. **Selenium paper (SS-610, 0.03 *M*), sample volume 38 cc.**

Such paper was prepared in several ways. The method finally adopted consists of soaking filter paper in a solution of potassium selenocyanate, allowing the paper to drain, and exposing it to hydrochloric acid vapors. The selenocyanate is decomposed by the acid, forming hydrogen cyanide and leaving potassium chloride and red selenium deposited on the paper. The steps of the procedure are the same as in the preparation of unbaked selenium sulfide paper, except for the difference in the reactions involved.

Potassium selenocyanate solution can be easily prepared by vigorously shaking potassium cyanide solution with excess pure selenium powder in a glass-stoppered flask. Formation of selenocyanate is nearly quantitative. The cyanide content of both the cyanide and selenocyanate solutions is conveniently determined volumetrically with standard silver nitrate in the presence of iodide. Selenocyanate solutions are stable when stored in glass-stoppered containers. They may be gravimetrically analyzed by precipitation of the selenium in acid solution (*6*).

The authors have either used 21.5-cm. circles or have cut 22 cm. × 16.5 cm. rectangles from large sheets of filter paper. The papers are soaked in selenocyanate solution for 2 minutes or more and are hung in a closed container to drain for 15 minutes. They are then introduced into an atmosphere of hydrogen chloride for 15 to 30 seconds, removed, and allowed to dry before being cut into strips. The hydrogen chloride atmosphere is obtained by bubbling tank anhydrous gas through concentrated hydrochloric acid, preferably with the aid of a sintered-glass type of gas washing bottle. It is important that the atmosphere be prepared before introducing the paper. Reaction is immediate and full development of color should be complete in a matter of seconds. Prolonged exposure (20 minutes) to the acid atmosphere results not only in a weaker, somewhat brittle paper, but also in detectable loss in reactivity of the deposited selenium. If the paper is allowed to air-dry after soaking in the selenocyanate solution, it still reacts immediately when placed in the hydrogen chloride atmosphere, but the selenium deposited under these conditions is not reactive to mercury vapor even at 200° C. The hydrogen chloride treatment should be carried out in a well ventilated hood. The outer margin of each sheet of sensitized paper is discarded, only the 15 × 13 cm. central rectangle being cut into strips 0.125 inch wide and 15 cm. long.

PROPERTIES OF SENSITIZED PAPERS

The characteristics of the various sensitized papers have been studied by passing standard samples containing mercury vapor over the paper in the form of strips 0.125 inch wide inserted in glass tubing of slightly greater inside diameter.

Except where otherwise noted, the mercury vapor sample has always been obtained by passage of standard ethylene-oxygen samples over mercuric oxide at 285° C. Usually the instrument described in the accompanying paper (*5*), or its equivalent, was used. The volume of sample was usually 38 cc. and the flow rate about 20 cc. per minute.

Choice of Paper and Flow Rate. Most of the authors' work has been done with Whatman No. 1 or Schleicher and Schuell (American) No. 610 filter paper. The latter is a very thin paper and usually showed a smaller difference between the lengths of blackening of the two sides of the strip. This difference seldom exceeds 1 mm. for the thinner paper. In the following discussion the sensitized paper used is identified by the symbol W-1 or SS-610 and the molar concentration of selenious acid or potassium selenocyanate used in preparing the paper.

The shape and sharpness of the boundary between the darkened and unreacted portions of a detecting strip are affected by the rate of flow of sample over the paper. However, the length of blackening of unbaked selenium sulfide paper (W-1, 0.05 *M*) at 125° C. was found to be unaffected when the flow rate was varied from 5 to 45 cc. per minute. A flow rate of 10 to 20 cc. per minute is recommended.

Effect of Paper Temperature. The length of blackening produced by a standard sample of mercury vapor on unbaked selenium sulfide paper is sensitive to the temperature at which the paper is used. On the other hand, the responses of baked selenium sulfide paper and selenium paper are comparatively insensitive to the temperature of usage if that temperature is not too low. Curves *A* and *B* of Figure 2 were obtained with selenium sulfide papers (W-1, 0.100 *M*) which differed only in that the former was baked and the latter was not. The baking process (30 minutes at 150° C.) removed much of the sulfur, so that at lower paper temperatures a longer length of baked paper was required to react with the standard sample of mercury. However, as the paper oven temperature was increased, sublimation from the unbaked paper increased, so that the difference in response of the baked and unbaked papers was comparatively small above 180° C. That sublimation of material from the strip occurs is evident from visible condensation of sulfur on the cool portion of the strip. When pure oxygen is passed over hot unbaked selenium sulfide paper in a long glass tube, sublimate forms where the gas reaches the cool portion of the tube. At higher paper temperatures the sublimate consists of a reddish deposit of selenium followed by a yellow deposit of sulfur. Curve *C* of Figure 2 was obtained with selenium paper (SS-610, 0.03 *M*). The concentration of mercury in the standard sample used for curves *A* and *B* corresponded to saturation at about 115° C., whereas a more dilute standard sample was used for curve *C*.

The response of three types of sensitized paper over a wider temperature range was studied by passing a 3-liter sample of oxygen saturated with mercury vapor at 26.9° C. over 0.125-inch-wide strips of the paper in a glass tube surrounded by a small cylindrical brass block oven at a flow rate of about 45 cc. per minute. The results are shown in Figure 3. Paper A was coated selenium sulfide paper supplied by the General Electric Co. Two strips of the paper with the uncoated sides back to back were used for each measurement. Paper B was unbaked selenium sulfide paper (W-1, 0.033 *M*), and C was selenium paper (SS-610, 0.080 *M*). All measurements were made at least in duplicate.

All three types of paper show a minimum length of blackening at about 65° C. The longer the length of blackening, the less

intense was the blackening, as is expected for deposit of a fixed amount of black mercuric sulfide or selenide over a varying area of paper. At temperatures above 65° C. the effect of sublimation is again marked with the selenium sulfide papers; it is even appreciable for the selenium paper at 175° with this large sample volume. At the flow rate used in these experiments, the rate of reaction below 65° was apparently too slow for complete removal of mercury vapor by the first unreacted sulfur or selenium which the sample encountered on the sensitized paper. In accordance with this explanation, it was found at these lower temperatures that the reacted portion of the strip was not uniformly darkened, the length of blackening was not well defined, and the length of blackening was sensitive to the flow rate.

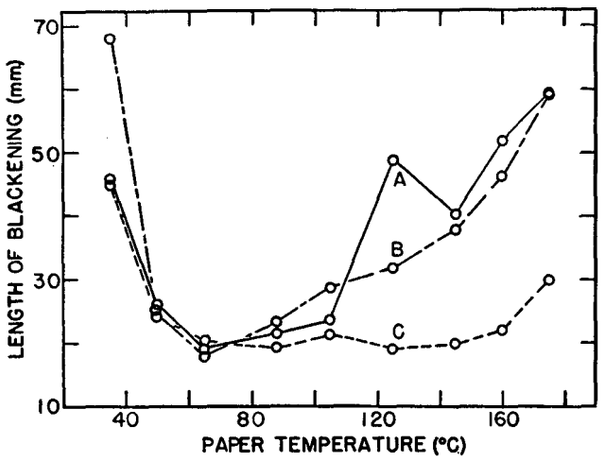

Figure 3. Effect of Temperature on Response of Sensitized Paper

A. Coated selenium sulfide paper
B. Unbaked selenium sulfide paper (W-1, 0.033 *M*)
C. Selenium paper (SS-610, 0.080 *M*)

The authors do not understand why a longer blackening was found at 125° than at 145° C. for the coated paper. (An average value of 49.0 mm. with a mean deviation of 2.4 mm. was found in five experiments at 125° performed at three different times, whereas the average value of 40.4 mm. with a mean deviation of 3.5 mm. was obtained from four experiments at 145°.)

Calibration Properties. The quantity of reactive material per unit area of sensitized paper may be adjusted by choice of paper thickness and concentration of selenious acid or selenocyanate solution used in its preparation. When the sensitized paper is used in the form of strips as described above, the width of the strip and the volume of sample used may also be adjusted to yield appropriate lengths of blackening for estimating mercury vapor at various concentrations. Of these various factors, the solution concentration is the easiest to vary over a wide range with satisfactory results. The lower limit to the solution concentration that may be used in preparing the paper is set by the degree of blackening required to produce perceptible contrast between the reacted and unreacted portions of a strip. This limit is approximately 0.002 *M* selenious acid or potassium selenocyanate.

The length of blackening of selenium papers (SS-610, 0.010 *M*, 0.030 *M*, 0.090 *M*) and of unbaked selenium sulfide papers (SS-610, 0.010 *M*, 0.030 *M*, 0.090 *M*) was determined as a function of mercury vapor concentration with the portable instrument for estimating ethylene in air (*5*). Ethylene-oxygen samples of known composition were made by preparing a standard mixture by use of a gas pipet and gasometer (*2*) and then diluting portions of this mixture with oxygen by use of a gas buret and a mixing bulb of known volume. In Figure 4 the observed lengths of blackening for some of these papers are plotted against the ethylene concentration of the sample. The values at zero ethylene concentration are blanks obtained with pure oxygen samples and result from dissociation pressure of the hot mercuric oxide. The mercury vapor concentration resulting from the oxidation of the ethylene is 5.4 times the ethylene concentration (*5*).

For the selenium papers, the length of blackening (corrected for blank value) was directly proportional to the ethylene content of the sample, the proportionality constant being inversely proportional to the concentration of selenocyanate used in preparing the paper. These results may be conveniently summarized by the equation

$$ac = K \qquad (1)$$

where a = length of blackening in millimeters per part per million of mercury per cubic centimeter of sample measured at 25° C.; c = molar concentration selenocyanate solution used; and $K = 1.26 \times 10^{-4}$ for 0.125-inch-wide SS-610 selenium paper strips. As the response of selenium paper is not sensitive to temperature in the range 65° to 200° for small volume samples, Equation 1 may be used for estimating the proper concentration of selenocyanate to use in preparing selenium paper for use in this temperature range. The corresponding value of K for W-1 selenium paper strips is 1.0×10^{-4}.

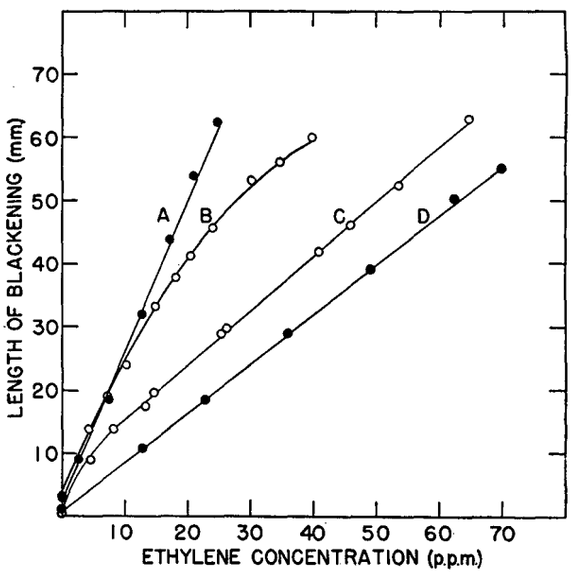

Figure 4. Calibration Properties of Sensitized Papers

Temp. 125° C. Sample volume 38 cc.
A, D. Selenium paper (SS-610, 0.010 *M*, 0.030 *M*)
B, C. Unbaked selenium sulfide paper (SS-610, 0.010 *M*, 0.030 *M*)
For curve *C*, ethylene concentration scale should be multiplied by three

The calibration curves for the unbaked selenium sulfide papers are not linear, as illustrated by curves *B* and *C* of Figure 4. In general, they consist of an initial portion of steep slope followed by a linear portion of lesser slope. The higher the concentration of selenious acid used in preparing the paper, the shorter is the initial region of higher slope. The nonlinearity is due to sublimation of sulfur from the first portion of the strip until the sample becomes saturated with sulfur vapor, thus leaving less active material to combine with mercury vapor in the first portion of the strip. For paper containing very small amounts of selenium sulfide, a major portion of the strip may be influenced by sublimation, as in curve *B* of Figure 4. On the other hand, for paper containing relatively large amounts of selenium sulfide, the initial region of high slope becomes very short and the calibration curve is linear over the concentration range for which it is useful. Because of the influence of the flow rate and volume of sample on sublimation effects, it is difficult to prepare unbaked selenium sulfide paper with reproducible calibration properties for use under conditions where the calibration curve is not linear in the mercury vapor concentration range of interest.

Uniformity and Reproducibility. The variations in response of strips cut from a single sheet of sensitized paper and of strips cut from different sheets of sensitized paper prepared by the same procedure at different times were studied by use of the instrument and standard samples. The main factors determining these variations are differences in paper thickness within a sheet and among different sheets, reproducibility of the experimental procedure in leaving a uniform deposit of active material, and variations of the widths of the paper strips. The widths of the paper strips employed showed a mean deviation from the average of about 1%. The lengths of blackening of strips cut from the same sheet of paper showed a mean deviation from the average of 2 to 3% when only the bottom ends or the top ends of the strips were used. The difference in response of bottom and top ends of strips from the same sheet was usually about 5%. The mean deviation from the average response of strips cut from different sheets processed in the same way was 3 to 5%. These figures were the same for selenium paper (0.03 *M*, SS-610), unbaked selenium sulfide paper (0.05 *M*, W-1), and baked selenium sulfide paper (0.05 *M*, W-1) when the baked papers were baked in a variable forced-draft oven in successive batches without changing conditions in the oven. All these figures were obtained with lengths of blackening of at least 20 mm.

Storage Properties. Selenium papers and both baked and unbaked selenium sulfide papers have been kept over a year at room temperature in the dark without change in their response to a standard sample of mercury vapor. In diffuse daylight both selenium and baked selenium sulfide papers tend to become a violet color over a period of months. Although this discoloration reduces the contrast between the unreacted and blackened portion after use, it does not seem to affect the response characteristics. It is recommended that the sensitized strips be kept in the dark in closed containers.

DISCUSSION AND CONCLUSIONS

Of the four types of paper considered, only the selenium paper possesses all the following properties: low sensitivity of response to temperature above 65° C., linear calibration, high degree of uniformity, high reproducibility in preparation, and satisfactory storage properties. Baked selenium sulfide paper is essentially similar to selenium paper, as most of the sulfur is removed in the baking process, but it lacks high reproducibility in preparation because of the sensitivity of its properties to the details of the baking process. Unbaked selenium sulfide paper lacks low sensitivity of response to temperature, whereas the coated paper seems also to have a lower degree of uniformity. Selenium paper is accordingly recommended for use under conditions of sample volume and temperature where appreciable volatilization of sulfur may be anticipated, such as estimation of mercury vapor formed when hot mercuric oxide is used as an oxidizing agent (*2, 5*). Either selenium paper or unbaked selenium sulfide paper should serve satisfactorily under conditions where volatilization of sulfur is negligible, as in the estimation of mercury vapor in atmospheres, although the resonance absorption photoelectric instrument of Woodson (*7*) is superior for this purpose.

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Determination of Trace Amounts of Selenium in Water

JACK L. LAMBERT[1], PAUL ARTHUR, AND THOMAS E. MOORE
Oklahoma Agricultural and Mechanical College, Stillwater, Okla.

SELENIUM, because of its toxicity and cumulative effect in the body, presents a health problem when it occurs in food or water in even minute amounts. It is the only element known to be taken up by plants from the soil in sufficient quantities to be toxic to animals (*10*). In natural waters, selenium may have its source in selenium-containing soils or rocks or in water draining from areas containing seleniferous vegetation. In such waters, it may occur as the selenite or selenate ion or as organically combined selenium in concentrations usually of the order of 1 p.p.m. or less. A concentration of 0.5 p.p.m. of selenium in water is considered potentially dangerous (*11*). This research was undertaken to develop a procedure for quantitatively determining selenium in whatever form it may occur in natural waters without first concentrating it.

REAGENTS

All reagents used were of the C.P. or analytical reagent grades, except the carbon dioxide, which was of commercial grade. Blank determinations, made to test the purity of the reagents, proved negative.

1. Sulfuric acid, 12 *N* (334 ml. of concentrated sulfuric acid per liter of solution).
2. Bromine, saturated aqueous solution.
3. Carbon tetrachloride.
4. Carbon dioxide, commercial cylinder.
5. Potassium bromide, saturated aqueous solution.
6. Sodium hypochlorite, 1%. Zonite, a readily available, carefully prepared, and stabilized 1% solution of sodium hypochlorite is recommended.
7. Sodium nitrite, saturated aqueous solution prepared fresh daily.
8. Urea, saturated aqueous solution prepared fresh daily.
9. Formic acid, 90%.
10. Phosphoric acid, 85%.
11. Tartaric acid, saturated aqueous solution prepared fresh daily. U.S.P. grade was substituted satisfactorily for the more highly purified grades in many determinations.
12. Cadmium iodide, 5.00% (5.00 grams in 100 ml. of solution). The cadmium iodide was dissolved in somewhat less than the necessary volume of water and the solution was boiled gently for 15 minutes to remove iodine, after which it was diluted to the correct volume and stored in a brown glass bottle.
13. The cadmium iodide–linear starch reagent (*1*) was prepared from twice recrystallized linear *A* fraction potato starch as described by Schoch and coworkers (*8, 9*). The crude *A* fraction was recrystallized from a hot aqueous solution saturated with 1-butanol, centrifuged, and finally dehydrated with successive portions of 1-butanol. The starch fraction so prepared dissolved easily and completely in boiling water.

[1] Present address, Department of Chemistry, Kansas State College, Manhattan, Kan.

To prepare the reagent, 11.00 grams of cadmium iodide were dissolved in 300 to 400 ml. of distilled water and the solution was boiled gently for 15 minutes, with water added to maintain an approximately constant volume. Enough distilled water was then added to increase the volume to about 800 ml., and 2.5 grams of the linear *A* fraction starch were added slowly with stirring to the gently boiling solution. The solution was filtered, if turbid, and the volume was brought up to 1 liter with distilled water.

This reagent is stable for long periods when stored in brown glass bottles and exposed only to ordinary diffuse sunlight (*1*). Cadmium iodide not only reduces the iodide ion concentration by complex ion formation and prevents photodecomposition to produce iodine, but it also, by its toxicity, prevents the growth of microorganisms. The use of ordinary "soluble" starches resulted in solutions that deteriorated within a relatively short time.

Reproducible color and intensity of color are thus obtainable with the reagent when it is used in the analysis of substances capable of oxidizing iodides to iodine. At low pH values, phosphoric, tartaric, oxalic, and maleic acids tend to stabilize the colloidal linear starch–iodine complex, while hydrochloric and sulfuric acids tend to cause precipitation. It is possible in the absence of an excess of strong mineral acids to obtain a very stable colloid. Because of its stability and versatility, the reagent is ideally suited as a shelf reagent for spectrophotometric procedures involving oxidizing agents in which interfering substances are removed or are absent from the solution.

14. Standard selenious acid solutions. Pure selenium dioxide was first prepared by the method described by Baker and Maxon (*2*), and further purified by sublimation in a stream of oxygen and nitrogen dioxide in a tubular sublimation apparatus similar to that described by Pitha (*7*). From this, a stock solution of selenious acid was prepared and found to contain 1066 ± 3 p.p.m. of selenium as determined by the gravimetric method (*4*). This solution was diluted to provide a second stock solution of 10.0 p.p.m. of selenium, from which the final solutions were made. The solutions so prepared were found to remain constant in concentration indefinitely.

APPARATUS

A small separatory funnel or a 25 × 200 mm. borosilicate glass test tube with a standard-taper No. 3 stopcock sealed to the bottom.

Borosilicate glass test tubes, 25 × 200 mm.

Pipets, 20.0, 10.0, 4.0, 2.0, and 1.0 ml.

Medicine dropper.

Instruments required for the determination of the iodine liberated by the oxidation of the iodide ion by selenious acid are (according to the method to be used):

Spectrophotometer, capable of measurements at 352 or 615 mμ, depending upon the method used. A Beckman Model DU quartz spectrophotometer was used in this research for precision measurements at both wave lengths, with 1.000-cm. matched Corex cells.

Ammoniacal Nickel-Chromate Visual Comparison Solutions were prepared by dissolving 19.75 grams of C.P. nickel ammonium sulfate hexahydrate, $Ni(NH_4)_2(SO_4)_2 \cdot 6H_2O$, and 0.036 gram of potassium chromate in sufficient 14% ammonium hydroxide solution (equal volumes of concentrated, 28 to 29%, ammonium hydroxide and water) to make 100.0 ml. of solution. This solution, which was 0.500 *M* with respect to the hexamminenickel(II) ion, $Ni(NH_3)_6^{++}$, was then carefully diluted with 7% ammonium hydroxide solution to prepare a series of standard solutions having the following molarities of the hexamminenickel(II) ion and having colors corresponding to the linear starch–iodine colors produced by the concentrations of selenium listed:

$Ni(NH_3)_6^{++}$, *M*	Concn. of Se, P.p.m.
0.085	1.4
0.072	1.2
0.060	1.0
0.048	0.8
0.035	0.6
0.022	0.4
0.010	0.2

A blank was included for comparisons at very low concentrations. The amount of chromate could be varied slightly, as any visual comparison of colors is largely subjective.

By making the dilutions with 7% ammonium hydroxide, the ammonium hydroxide concentration was kept approximately constant and independent of the concentration of the hexamminenickel(II) ion. The solutions should be kept sealed tightly in 25 × 100 mm. borosilicate glass test tubes fitted with rubber toppers, to which a coat of collodion has been applied at the ubber-glass connection. It was found convenient to have the volume of the standard comparison solution approximately equal to that of the unknown solution compared to it—i.e., about 20 ml. On standing, a slight precipitate was often apparent, but checks made by visual comparison and by measurement with the Beckman DU spectrophotometer showed no significant changes in the colors of these solutions. Although the solutions appeared to be stable to light, they should not be exposed to direct sunlight for long periods of time.

A Confined Spot Apparatus such as that described by the authors (*5*).

PROCEDURE

This procedure is recommended for use if the surface and ground waters have a low content of organic matter and a normal content of dissolved inorganic salts, especially iron. Waters having a high concentration of the substances mentioned above or having excess turbidity and irremovable color, or to be analyzed by the "confined spot" method, should be put through the distillation process described under the Distillation Process for Unusual Waters. The analysis is carried out in the following manner:

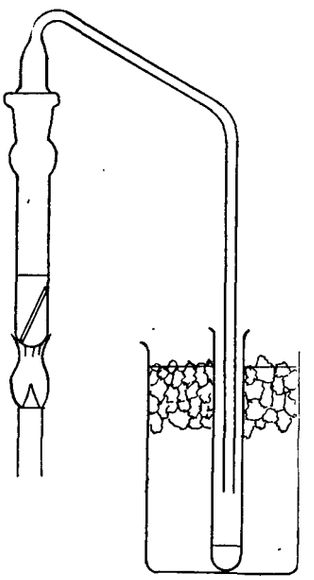

Figure 1. Distillation Apparatus

1. Pipet a 20.0-ml. sample of water into a separatory funnel.
2. Add 4.0 ml. of 12 *N* sulfuric acid.
3. Add 4 drops of the saturated bromine solution and pass in carbon dioxide at a moderate rate for 2 or 3 minutes. Add enough bromine to give a brown color to the first extraction with carbon tetrachloride.
4. Extract both the excess bromine and the iodine formed from any iodide originally present with approximately 5-ml. portions of carbon tetrachloride. Maintain agitation with a stream of carbon dioxide gas during the extraction. Three extractions are usually sufficient.
5. Pipet 10.0 ml. of the solution into a 25 × 200 mm. borosilicate glass test tube. (If iodide is known to be essentially absent, as it is in most natural waters, steps 1, 2, 3, and 4 may be omitted and the determination begun at step 5 with a 10.0-ml. sample. Add 2.0 ml. of 12 *N* sulfuric acid and proceed to step 6.)
6. Add 1.0 ml. of saturated potassium bromide solution and 1.0 ml. of Zonite, stopper the test tube loosely with a glass stopper, and heat on a gently boiling water bath for 1 hour.
7. Add 1.0 ml. of saturated sodium nitrite solution and pass in carbon dioxide at a moderate rate for 10 minutes.
8. Add 1.0 ml. of saturated urea solution and wash down the walls of the test tube by carefully increasing the rate of flow of the carbon dioxide for a few seconds.
9. Add 1.0 ml. of saturated tartaric acid solution, 1.0 ml. of 85% phosphoric acid, 1.0 ml. of 12 *N* sulfuric acid, and 2.0 ml. of 90% formic acid. Pass in carbon dioxide gas for 10 minutes after adding the formic acid.
10. **Option A.** Add 1.0 ml. of the 5.00% cadmium iodide solution, pass in carbon dioxide at a very slow rate for 5 minutes, and determine the optical density at 352 mμ using a spectrophotometer.

Option B. Add 1.0 ml. of the cadmium iodide–linear starch reagent and pass in carbon dioxide at a very slow rate for 5 minutes. The resulting solution can be compared to the visual comparison standard solutions, or its optical density can be determined in the spectrophotometer at 615 mμ.

It was found that the optical densities in either option could be determined using distilled water as a reference. In extreme cases, it was necessary to use as the blank a solution prepared by put-

The research described was undertaken to develop a method for the determination of trace amounts of selenium in natural waters. The method transforms all forms of seleniferous matter into selenious acid, which then quantitatively oxidizes iodide to iodine. The liberated iodine is determined colorimetrically in one of four ways: (1) as I_3^- using its absorption at 352 mμ; (2) by means of the color produced with linear *A* fraction starch, using its absorption at 615 mμ; (3) by comparison of the linear *A* fraction starch-iodine color with standards made from ammoniacal nickel sulfate–potassium chromate solutions; and (4) by deliberate coagulation of the starch-iodine complex and filtration to give a quantitative spot test. The method is useful for concentrations from 0.1 to 5.0 p.p.m. of selenium, and provides for removal of all probable interferences. Cadmium iodide, which was found to have excellent keeping qualities, was used throughout as a source of iodide ions. This method can be applied to most natural waters without preliminary evaporations and can determine selenium in the low concentrations which have been found to be of significance in water analysis.

ting the natural water through all the steps in the procedure except the one involving the addition of iodide.

Curves representing the relationship between optical densities and concentrations of selenium were prepared using solutions of known selenious acid concentration for both of the spectrophotometric methods given. The effective range of concentrations using the 352 mμ method was from 0.1 to 5.0 p.p.m. of selenium, while the range of the method at 615 mμ was from 0.1 to 1.4 p.p.m. of selenium. Above 1.4 p.p.m. of selenium, serious coagulation of the starch-iodine complex tended to alter both the hue and the intensity of color of the solution. In general, results with solutions indicating concentrations below 0.1 p.p.m. of selenium should be considered of doubtful validity.

DISTILLATION PROCEDURE FOR UNUSUAL WATERS

For waters having organic matter, iron, dissolved salts, or irremovable color in abnormally large amounts, it was found that a distillation step should be included immediately following step 6 of the procedure. The remaining steps in the procedure were then carried out using the distillate from this step. This modification is recommended, furthermore, for samples that have been concentrated by evaporation, as these are likely to contain much dissolved material.

The distillation apparatus is shown in Figure 1.

It included a standard-taper 24/40 joint that had been sealed to a length of a 25-mm. diameter borosilicate glass test tube to make a test tube 25 × 200 mm., and the condenser illustrated. This tube was used also for the digestion treatment on the water bath, during which time it was loosely stoppered with a standard-taper 24/40 stopper. A capillary boiling tube or a piece of clay plate was added to prevent bumping, and the solution was distilled rapidly into a 25 × 200 mm. test tube containing 2.0 ml. of 12 *N* sulfuric acid. A sufficiently quantitative distillation was obtained if the solution was boiled until essentially only sulfuric acid remained in the distillation tube. It was not necessary to heat until fumes of sulfur trioxide appeared. The use of the jacketless condenser prevented condensation of the vapor until it reached the ice-cooled receiving tube. If the bromine and selenium tetrabromide were allowed to react in a neutral or nearly neutral solution, oxidation to the selenate would occur; this, however, is prevented by the acid in the receiver.

Although some bromine was lost during the distillation, little water was lost, and the amount of selenium escaping was small.

Table I. Recoveries Obtained Using Distillation Procedure

Selenium Added, P.P.M.	Selenium Recovered, P.P.M.
5.0	5.0
2.5	2.7
1.4	1.15
1.4	1.45
1.0	0.8
1.0	1.25
0.7	0.4
0.6	0.3
0.2	0.1

This was shown by the recoveries tabulated in Table I, which were determined by the method described in step 10, Option A, of the procedure.

These data indicated that while the percentage errors were greater (particularly in the lower concentrations) if the distillation procedure was employed, the accuracy was still well within the allowable limits for analyses of this kind. Greater accuracy was obtained when a new calibration curve was constructed from standard selenious acid solutions which also had been subjected to the distillation procedure.

Confined Spot Method. Strong mineral acids tend to coagulate the linear starch–iodine complex. If interferences are absent or are removed by the distillation procedure, this phenomenon can be made the basis for a highly sensitive quantitative determination for selenium. The procedure is as described above, except that tartaric acid is omitted in step 9. When the linear starch–cadmium iodide reagent has been added, the solution is filtered through Whatman No. 50 filter paper impregnated with barium sulfate in the confined spot apparatus described by the authors (*5*). Comparison of the spots so obtained with similar spots prepared from known samples permits the ready determination of selenium in concentrations from 0.03 to 0.20 p.p.m. in intervals of 0.03 p.p.m.

The chief difficulty encountered with this method is its extreme sensitivity, which renders it unusually susceptible to interference from any oxidizing agents that might be present in extremely minute quantities.

DISCUSSION OF METHOD

Chemistry of the Method. The basic chemistry of the method involves the oxidation or reduction of selenium, both inorganic and organic, to selenious acid and the quantitative oxidation of iodide ion by the latter to elementary iodine. Bromine–hydrobromic acid mixtures were found satisfactory for the conversion of the selenium, as they act rapidly and the excess bromine is easily destroyed by nitrous acid. The last traces of nitrous acid are removed by urea, which is without effect upon the selenious acid or liberated iodine.

Interferences. Substances most likely to interfere with the method are oxidizing agents and reducing agents which might occur in water in equal or greater concentration than that of the selenium, particularly if the water comes from an industrialized area. Oxides of sulfur and nitrogen, chlorine and chloramines, nitrates, and compounds of manganese and chromium did not interfere after treatment with the reagents used in the procedure. The elimination of these substances depended, for the most part, on their reaction with the reagents, bromine, hydrobromic acid, and nitrous acid.

Dissolved oxygen was readily displaced by the stream of carbon dioxide, and the solution was effectively protected from oxygen by the carbon dioxide layer above. Iodine liberated from iodides was removed by extraction with carbon tetrachloride.

Iron was first oxidized to the ferric state and then converted by the phosphoric and tartaric acids to very stable colorless complexes incapable of oxidizing the iodide. The iron in freshly prepared solutions of ferric salts in water reacted with the phosphoric acid, forming a complex that was incapable of oxidizing the iodide ion in acid solution of the pH used in this procedure. Iron solutions that had been allowed to hydrolyze to brown colloidal ferric hydroxide or hydrated oxides did not react readily with phosphoric acid, but the iron in such solutions did react at an appreciable rate with tartaric acid to form a stable complex.

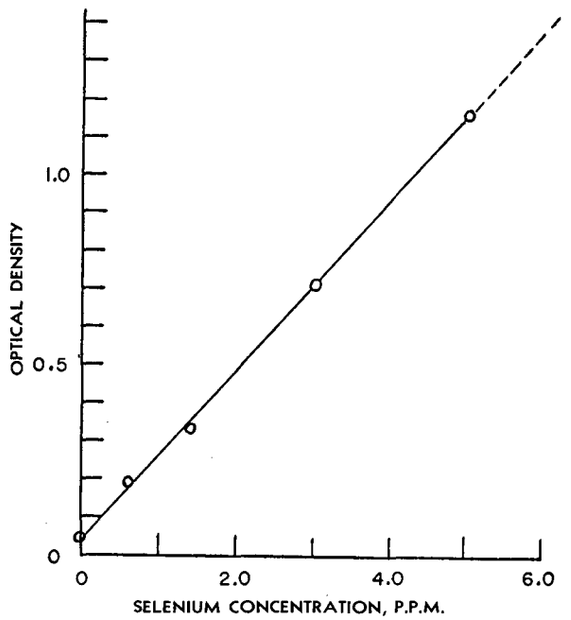

Figure 2. Optical Densities of Triiodide Solutions Produced by Selenious Acid Solutions of Known Concentrations
At 352 mμ

Although arsenic is converted to the arsenate by the oxidation steps described, it was found that formic acid is a specific reducing agent for arsenic acid in the presence of selenious acid. As this reduction is not instantaneous, arsenic acid concentrations of the order of 2.0 p.p.m. are completely reduced, but extremely high concentrations—e.g., 100 p.p.m.—are not entirely eliminated in the time allowed in the procedure.

Commonly encountered ions, such as calcium, magnesium, aluminum, sulfate, and chloride, do not influence the results if present in normal amounts. For waters of exceptionally high dissolved salt concentration, the distillation previously referred to should be followed.

In addition to the oxidation of the lower states of selenium, discussed above, the problem of reducing any selenate originally present called for quantitative study. To determine whether the procedure could be used if the selenium were present as selenate, samples of a solution containing 1.0 p.p.m. of selenium in the form of selenic acid were subjected to several different treatments. The results of varying the conditions in the procedure to convert the selenate are summarized in the following:

1. 10.0 ml. of the 1.0 p.p.m. selenium solution plus 2.0 ml. of 12 *N* sulfuric acid plus 1.0 ml. of saturated potassium bromide solution, boiled for 10 minutes; 70% conversion.
2. Same procedure as in (1) but with 1.0 ml. of Zonite in addition, followed by heating on the water bath for 45 minutes: 78% conversion.
3. Same procedure as in (2) but heated on the water bath for 1 hour: 88% conversion.

This indicated that at least 1 hour is required for the conversion. Because prolonged heating might result in appreciable loss of the volatile selenium tetrabromide, a longer heating period is not advised.

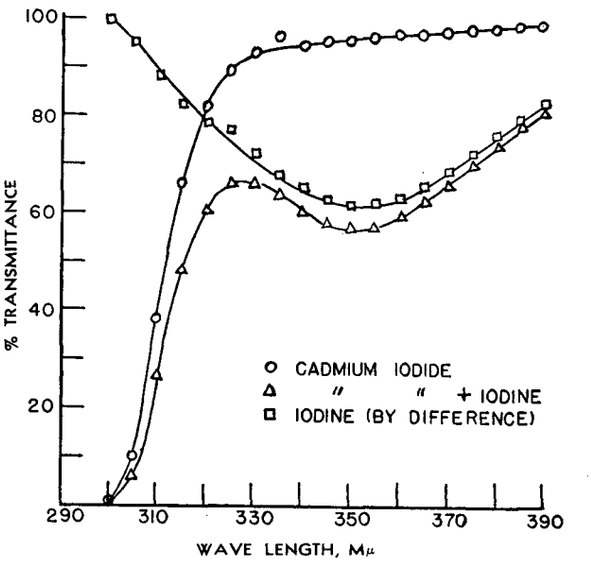

Figure 3. Transmittance of Cadmium Iodide Solution Alone and with Added Iodine

Table II. Analyses of Artificially Prepared Samples
(In p.p.m.)

Sample No.	No. of Detns.	Concn. Found (Average)	Actual Concn.	Average Deviation	Maximum Deviation	Total Solids in Water Used
1	5	3.56	4.00	0.25	0.44	556
2	5	2.21	2.15	0.09	0.14	556
3	5	0.222	0.150	0.020	0.043	184
4	2	0.148	0.150	0.012	0.013	184

EXPERIMENTAL RESULTS

Determination Using Triiodide Ion. The 352 mμ absorption peak of the triiodide ion can be employed to detect the iodine liberated by the selenious acid oxidation of cadmium iodide. A Beckman DU quartz spectrophotometer was used with a tungsten filament light source, which is satisfactory at 352 mμ (*10*). Iodide ion was provided by the 5.00% cadmium iodide solution, described in Option A of step 10 of the procedure; an accurately prepared solution is necessary because of the absorption, small but appreciable, exhibited by cadmium iodide solutions at 352 mμ. A linear relationship between the selenious acid concentration and the optical density of the triiodide solutions was found (see Figure 2) for concentrations up to 5.0 p.p.m. of selenium; above this, however, precipitation of elementary selenium affected the results.

The small but definite value of the intercept on the optical density axis is due to the slight absorption of cadmium iodide at 352 mμ as compared to distilled water as a reference. As the cadmium iodide concentration is held essentially constant, however, its absorption remains the same at all triiodide ion concentrations. Figure 3 illustrates the transmittance curve of a cadmium iodide solution and of the same solution with iodine added.

A number of determinations were carried out on artificially prepared samples, made by adding selenious acid solution to natural surface waters whose compositions were known to be

representative of this type of water (from routine analyses performed by the Water Resources Laboratory, Geological Survey, U. S. Department of the Interior, Stillwater, Okla.). Typical results are tabulated in Table II.

Table III. Comparison of Analyses Using Cadmium Iodide–Linear Starch Reagent

(In p.p.m. of selenium)

No. of Detns.	Known Concn.	Visual Comparison Av. results	Visual Comparison Av. deviation	Beckman DU, at 615 mμ Av. results	Beckman DU, at 615 mμ Av. deviation
3	1.4	1.43	0.09	1.42	0.15
8	1.0	0.94	0.15	0.92	0.09
5	0.6	0.56	0.06	0.60	0.06
4	0.2	0.20	0.00	0.20	0.02

Samples 1, 2, and 3 were analyzed without the use of tartaric acid as a complexing agent for ferric iron. At the higher concentrations of selenious acid, the effect of the iron present was insignificant, but at the very low concentrations, iron caused appreciable error. Accordingly, sample 3 was re-analyzed using tartaric acid, and much better results were obtained, as shown by sample 4 in Table II.

In each of the artificially prepared water samples analyzed, the surface water used in the preparation of the sample was taken from a nonseleniferous area and, therefore, was assumed to be selenium-free: however, in one sample, a large discrepancy was found between the observed selenium content (0.150 and 0.192 p.p.m.) and the amount of added selenium (0.066 p.p.m.). In this case, the surface water was found to have 0.10 p.p.m. of selenium already present, and because this was of approximately the same order of magnitude as the selenium added, it introduced a large percentage error. A sample taken from the same location at a later time showed 0.12 p.p.m. of selenium.

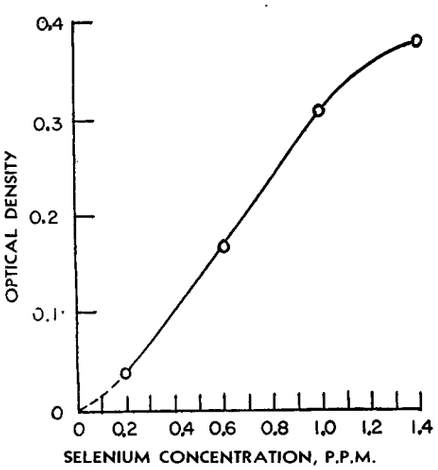

Figure 4. Optical Densities of Linear Starch–Iodine Complex Produced by Selenious Acid Solutions

At 615 mμ

Determination by Linear Starch–Iodine Method. The linear starch–iodine blue complex can be determined either spectrophotometrically at 615 mμ or by use of visual comparison standards. The plot of optical density against concentration of selenious acid again is a straight line for the most part, but the relation becomes uncertain above 1.4 p.p.m. of selenium because of coagulation of the linear starch–iodine complex (see Figure 4). Transmittance curves of three concentrations of the linear starch–iodine complex are shown in Figure 5.

A comparison of the spectrophotometrically determined amounts of selenium with the amounts found employing the ammoniacal nickel-chromate visual comparison solutions is given in Table III. This comparison shows clearly that both methods are satisfactory in the range 0.1 to 1.4 p.p.m. of selenium. The values listed in the fifth and sixth columns were measured with a Beckman DU spectrophotometer.

Two samples of naturally seleniferous water and one synthetically prepared sample (sample 2 in Table II) were analyzed by the linear starch–iodine method. The values reported for the natural samples were supplied by the laboratories of the University of Wyoming and were obtained by standard methods for the analysis of selenium.

The agreement between the sets of data (Table IV) is seen to be satisfactory. In the cases of the 9.0 and 2.15 p.p.m. selenium samples, a preliminary dilution was necessary before applying the new method of analysis.

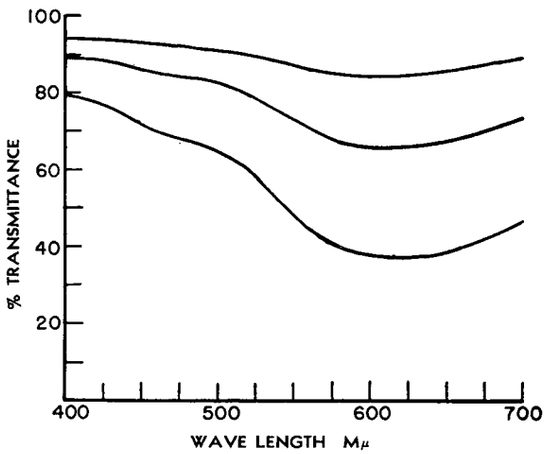

Figure 5. Transmittance of Linear Starch–Iodine Complex at Three Concentrations

Table IV. Analyses of Natural and Artificial Seleniferous Waters Using Cadmium Iodide–Linear Starch Reagent

(In p.p.m.)

Sample	Reported	Determined Visual	Determined Beckman
1	0.25	0.2	0.28
1	0.25	..	0.17
2	9.0	8.0	8.2
2	9.0	..	8.8
3	2.15 (synthetic)	..	2.32

ACKNOWLEDGMENT

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Mention should also be made of the aid and interest taken in this problem by I. W. Walling and A. M. Schnitzer of the Water Resources Laboratory, Geological Survey, U. S. Department of the Interior, located at Stillwater, Okla.; of the gifts of crude linear *A* fraction starch by T. J. Schoch of the Corn Products Re-

fining Co., Argo, Ill.: and of the samples of naturally seleniferous waters supplied by O. A. Beath of the University of Wyoming.

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Determination of Gamma Isomer Content of 1,2,3,4,5,6-Hexachlorocyclohexane

EMORY E. TOOPS, JR., AND JOHN A. RIDDICK
Commercial Solvents Corp., Terre Haute, Ind.

A simple, rapid, and direct method for determining the gamma isomer content of lindane was needed for production control and specification analysis. The freezing point of a purified sample of the gamma isomer and its cryoscopic constant were determined with a platinum resistance thermometer. A calculated freezing point of 112.86° C. was obtained for 100.00 mole % gamma isomer. The application of the freezing point depression method for the routine analysis of the gamma isomer content of lindane was checked on synthetic mixtures. The apparatus consisted of a simple freezing cell and a mercury thermometer specially constructed to the authors' specifications. The method has an accuracy of about ±0.05 mole %.

THE name "lindane" has been coined and accepted for the insecticide containing not less than 99% by weight of γ-1,2,3,4-5,6-hexachlorocyclohexane (γ-benzene hexachloride) (*4*). Although there are several methods of analyzing mixtures of the isomeric hexachlorocyclohexanes (*1, 5, 8, 9*), there are no published methods for lindane. A suitable method should be not only direct and accurate but rapid enough for production control and specification purposes.

In the opinion of the authors, a freezing point depression method fulfills the above requirements. Such a method requires only modest equipment and can be used by any intelligent technician.

There is a wide variance among the reported values of the melting or freezing point of the gamma isomer. Some work has been done in evaluating the cryoscopic constant and applying it to the determination of the gamma isomer content in technical benzene hexachloride (*3*). However, in the absence of a reliable freezing point it was decided that a complete redetermination was advisable.

The procedure used for determining the thermodynamic freezing point of the gamma isomer was based on the method used at the National Bureau of Standards by Rossini and coworkers (*6, 11*) for hydrocarbons.

The basic thermodynamic relationship (*11*) between the temperature of equilibrium and the composition of the liquid phase, or solvent, for an equilibrium mixture consisting of a liquid phase containing two or more components and a crystalline phase containing one of these components is:

$$-\ln N_1 = -\ln(1 - N_2) = \frac{\Delta H_f^\circ}{Rt_f^{\circ 2}}(t_f^\circ - t)\left[1 + \left(\frac{1}{t_f^\circ} - \frac{\Delta C_p}{2\Delta H_f^\circ}\right)(t_f^\circ - t) + \ldots\right] \quad (1)$$

where

R = gas constant per mole
t_f° = absolute temperature of freezing point of major component when $N_2 = 0$
ΔH_f° = heat of fusion per mole of major component at temperature t_f°
ΔC_p = heat capacity per mole of pure liquid minus pure solid for major component in pure state at temperature t_f°
t = given temperature of equilibrium

DETERMINATION OF THERMODYNAMIC FREEZING POINT

The freezing cell assembly used for the determination of the freezing point of the gamma isomer was essentially that developed by Mair, Glasgow, and Rossini (*11*).

For work at higher temperature 20 turns of No. 20 Nichrome wire were wound on the outside of the cell and the entire unit was enclosed in an outer glass jacket (Figure 1). The temperature around the freezing cell, and hence the thermal head, was controlled by a Variac connected to the Nichrome heater. The cooling rate was controlled by varying the pressure in the vacuum jacket. The temperature between the freezing cell and the outer jacket was measured with an iron-constantan thermocouple.

The freezing temperatures were measured with a four-junction platinum resistance thermometer using a Leeds & Northrup No. 8069 Mueller bridge. All temperatures were measured with a resistance thermometer calibrated by Leeds & Northrup against a National Bureau of Standards certified resistance thermometer.

Preparation of High Purity γ-1,2,3,4,5,6,-Hexachlorocyclohexane. Commercial grade lindane was purified by the following method.

Recrystallization from acetone, using 1 ml. of solvent for each gram of lindane.

Recrystallization from fractional distilled iso-octane (2,2,4-trimethylpentane), using 4 ml. of solvent for each gram of lindane.

Recrystallization from redistilled Phillips commercial grade *n*-hexane, using 4 ml. of solvent for each gram of lindane.

All recrystallizations were carried out by heating to effect complete solution, then rapidly cooling the hot solution in an ice bath to obtain a slurry of fine crystals. The product ob-

tained from each step in the recrystallization was separated by filtration and air-dried. The final air-dried material was further dried at 60° C. and 1-mm. pressure for 24 hours.

The over-all recovery from the first two recrystallizations was 60 to 65%. A 90% recovery was obtained from the third recrystallization.

The purified gamma isomer was found to hold adsorbed moisture very tenaciously and complete removal by oven drying under reduced pressure was difficult. This adsorbed moisture and any residual solvent were removed by dispersing dry nitrogen through the molten material, held at 115° C. for 2 hours, just prior to determining the freezing point. The flow of nitrogen was adjusted just to fill the melt with fine bubbles without violently agitating the surface.

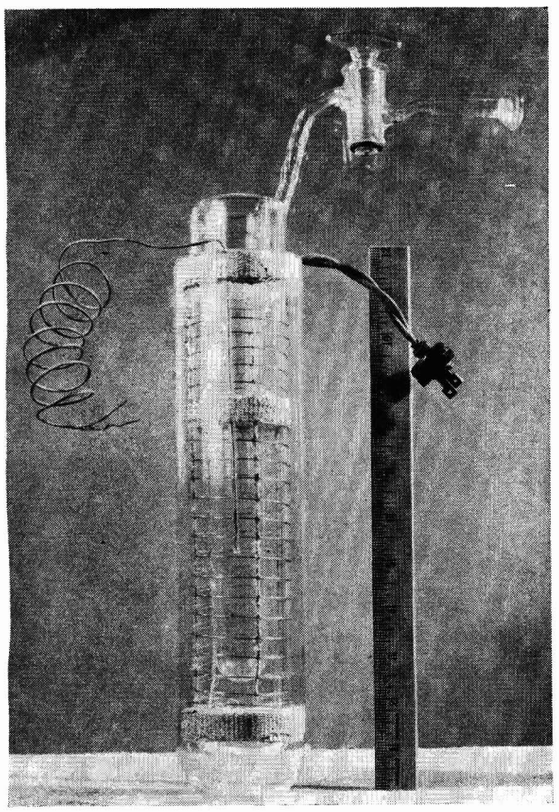


Figure 1. Freezing Cell

Procedure. Time-temperature freezing curves were determined using 70 grams of purified gamma isomer. Stirring was maintained constant at 80 strokes per minute. Time-resistance readings were recorded for intervals of 0.05 ohm (ca. 0.5° C.) while the melt was cooling to establish the cooling rate. The optimum rate for the purified gamma isomer was found to be about 0.1 ohm every 1 to 1.5 minutes (ca. 1° C. every 1 to 1.5 minutes) when the temperature was still 7° above the freezing point. At the appropriate time the solution was seeded to prevent severe undercooling.

With the onset of crystallization, time-resistance measurements were recorded for intervals of about 1 minute until the stirrer began to labor.

A preliminary plot was then made of the time-resistance data to establish zero time (the time at which crystallization would have begun in the absence of undercooling). For this plot the time scale was taken so that 1 cm. was equivalent to 1 minute and on the resistance scale 1 cm. was equivalent to 0.2 ohm (ca. 0.2° C.). If the extent of undercooling is small, the equilibrium portion of the curve may be extrapolated back to the liquid cooling line to obtain zero time. This type of curve is shown in Figure 2.

In order to locate accurately the resistance corresponding to the freezing point, the time-resistance observations were plotted with 1 cm. on the time scale equivalent to 1 minute and 1 cm. on the resistance scale equivalent to 0.0001 to 0.0002 ohm (ca. 0.001 or 0.002° C.). The equilibrium portion of the curve was then extrapolated back to the zero time line to obtain the resistance corresponding to the thermodynamic freezing point.

If the extent of undercooling is great, the resistance at zero time is more accurately determined by the geometric method described by Mair, Glasgow, and Rossini (*11*). The mathematical derivation of this relationship and the proof of the geometrical solution are discussed by Taylor and Rossini (*12*).

The resistance corresponding to the freezing point for zero impurity ($t_f°$) was also determined by the geometric method of Taylor and Rossini. The precision in determining $t_f°$, however, depends to a great extent on the time-temperature observations extending over a large fraction (about the order of one fourth to one half) of the material crystallized. Because of the nature of the gamma crystals, it was impossible to crystallize more than about one sixth of the melt before the laboring of the stirrer introduced enough heat to cause a rise in temperature. This is apparent in Figure 2 just before the stirrer was stopped. The precision in determining the actual freezing point, t, of the gamma isomer is nearly as good as the precision in determining individual temperatures on the equilibrium portion of the curve.

For calculating the purity of a given compound from freezing point data, it is more convenient to transform Equation 1 to the form:

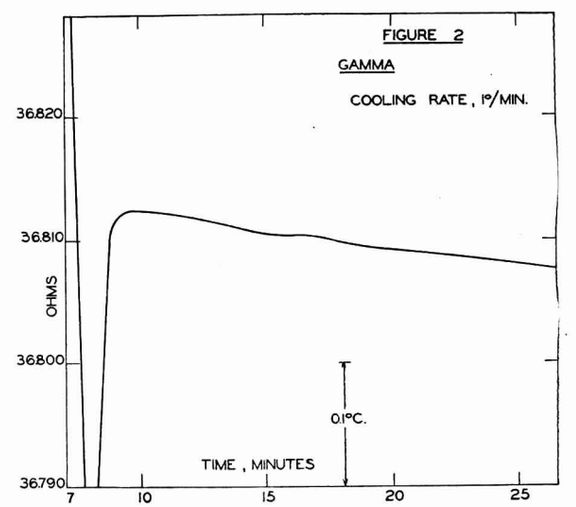
$$\log_{10} P = 2.00000 - \frac{A}{2.30259}(t_f° - t)[1 + B(t_f° - t) + \ldots] \quad (2)$$

where

A = cryoscopic constant (mole fraction per 1° lowering) = $\frac{\Delta H_{tf°}/Rt_{f°}^2}{}$

$B = \left(\frac{1}{t_f°} - \frac{\Delta C_p}{2\Delta H_f°}\right)$

P = mole % purity



If the purity of the sample is high—i.e., $(t_f° - t)$ is small—the second cryoscopic constant, B, may be neglected without significant error and Equation 2 reduced to:

$$\log_{10} P = 2.0000 - \frac{A}{2.30259}(t_f° - t) \quad (3)$$

Since $A = \Delta H_{tf°}/Rt_{f°}^2$, it was necessary to know the heat of fusion. In the absence of calorimetric data, the heat of fusion was approximated from Walden's rule (*7*) to be 4900 calories per mole.

Table I. Freezing Points of Purified γ-1,2,3,4,5,6-Hexachlorocyclohexane

Sample No.	Freezing Point, ° C.	Calcd. F.P. for Zero Impurity, ° C.	Calculated Purity, Mole %
1	112.839	112.866	99.95
2	112.842	112.862	99.97
3	112.838	112.863	99.96
4	112.830	112.857	99.95
5	112.837	112.869	99.96
Mean	112.837	112.863	99.96

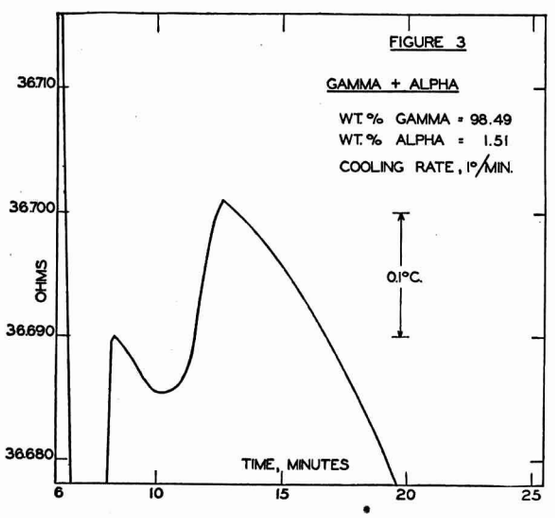
Table II. Determination of Cryoscopic Constant

Sample No.	Mole Fraction Gamma Isomer	Freezing Point, ° C.	Solute	A, Mole Fraction/1° Lowering
1	0.9946	112.481	Alpha isomer	0.0141
2	0.9897	112.141	Alpha isomer	0.0143
3	0.9897	112.149	Alpha isomer	0.0145
4	0.9896	112.180	Beta isomer	0.0153
5	0.9896	112.176	Alpha isomer	0.0156
6	0.9893	112.111	Naphthalene	0.0156
7	0.9838	111.825	Phenanthrene	0.0157
8	0.9772	111.492	Naphthalene	0.0168
9	0.9696	111.025	Alpha isomer	0.0168
10	0.9505	109.862	Alpha isomer	0.0169
11	0.9335	108.890	Alpha isomer	0.0173
12	0.8891	106.228	Alpha isomer	0.0177

The freezing point data for the purified gamma isomer are summarized in Table I.

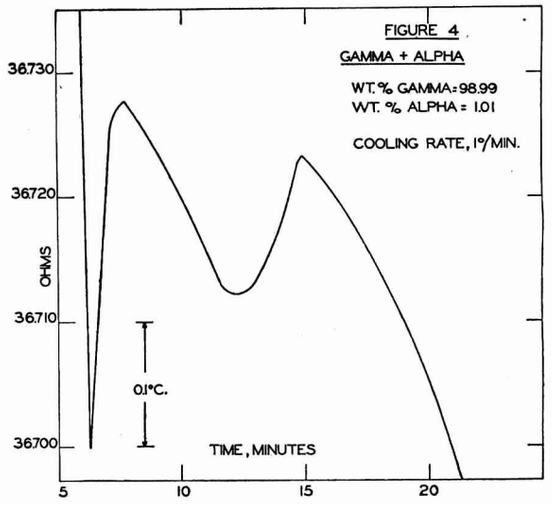
DETERMINATION OF CRYOSCOPIC CONSTANT

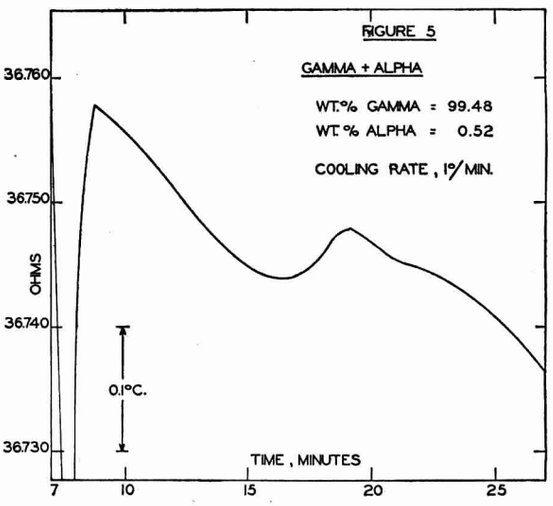
The cryoscopic constant was calculated by Equation 3 from the freezing points of a number of synthetic samples ranging from 88.91 to 99.46 mole % gamma isomer.



The synthetic mixtures were prepared by accurately weighing the desired amount of solute into the freezing cell. The purified gamma isomer was then weighed into the cell, the mixture melted, and the time-temperature freezing curve determined in the manner previously discussed.

The purified gamma isomer was dried by passing dry nitrogen through the melt for 2 hours. The melt was then poured on a stainless steel sheet, flaked, and powdered. It showed no surface adsorption of moisture when placed in humidistats ranging from 30 to 91% relative humidity for 7 days. X-ray diffraction patterns showed that rapid solidification caused no apparent change in crystal structure.





The freezing points of the synthetic samples, the solutes used, and the calculated values of the cryoscopic constant are given in Table II.

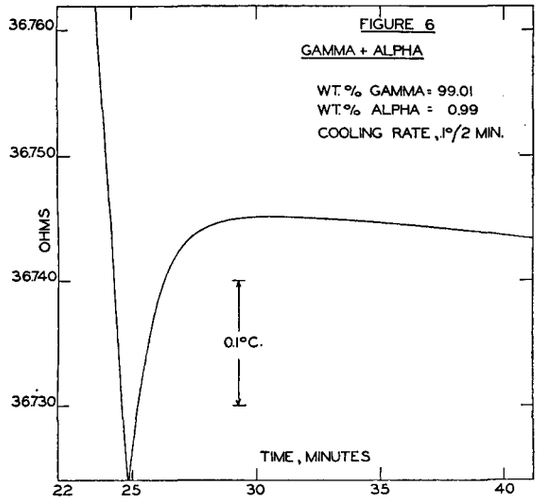
Only the value of the first cryoscopic constant, A, was calculated. In the absence of accurate calorimetric data no attempt was made to evaluate the second cryoscopic constant, B. The variation in the value of A, shown in Table II, is to be expected, as the limiting form of the freezing point lowering equation was used.

Table III. Analysis of Known Samples of Gamma Isomer

Sample No.	γ-Isomer Concentration, Mole % Calculated	Found
1	99.58	99.53
2	99.44	99.44
3	99.30	99.23
4	98.99	98.95
5	98.97	99.03
6	98.82	98.84
7	98.74	98.75
8	98.35	98.40
9	98.11	98.23

Lindane is by definition at least 99% by weight of the gamma isomer. Therefore the value of A used should be the best value determined for the concentration range, 99 to 100%. The value of A used for the analytical method was the mean of the first five values in Table II, or 0.0148 mole fraction per 1° lowering of the freezing point. Should it be desired to analyze samples of the gamma isomer containing more than 1% and up to 11% of the other isomers, the appropriate value of A may be obtained from Table II.

According to fusion data (*2*, *10*) there is evidence that the gamma isomer can exist in three polymorphic forms. In determining the cryoscopic constant additional evidence was obtained that polymorphism can exist.



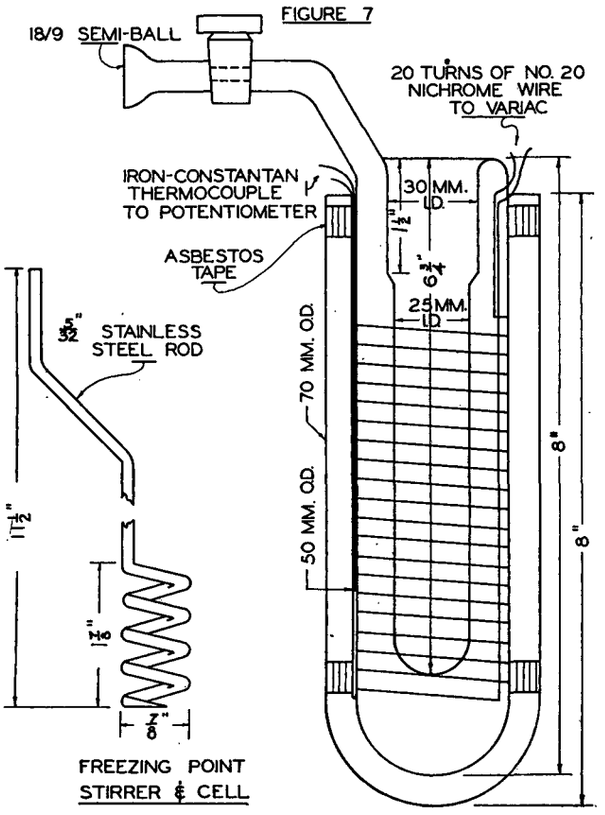


Table IV. Effect of Bubbling Nitrogen through α and γ Mixtures

Sample No.	Nitrogen Treatment Time, Hours	γ-Isomer Concentration, Mole %	
		Calculated	Found
1	0.0	98.99	98.95
2	0.5	98.96	98.94
3	1.0	98.92	98.87
4	1.5	98.96	98.97
5	2.0	98.96	98.99

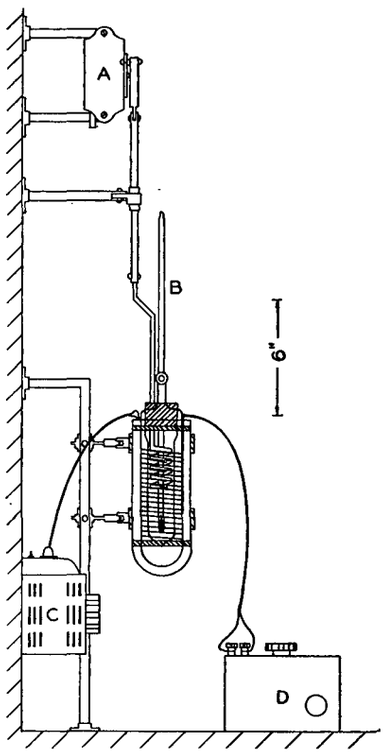


Figure 8. Routine Freezing Point Assembly

A. Windshield wiper stirrer, 60 strokes per minute
B. Thermometer
C. Variac, to control cell heater
D. Potentiometer, direct reading iron-constantan

The first determinations of the cryoscopic constant were made using the alpha isomer as the solute and a cooling rate of about 1° per minute. For a gamma isomer concentration of about 97.8 to 99.8 mole %, this fast cooling rate gave time-temperature curves with two maxima. Three of these curves are illustrated in Figures 3, 4, and 5. However, when the cooling rate was lowered to 1° every 2 to 3 minutes, normal time-temperature resulted (Figure 6). For the same concentrations, the temperature of the second maximum corresponded with the temperature obtained from a normal type curve. As yet no further work has been done on the polymorphic forms of the gamma isomer.

DETERMINATION OF GAMMA ISOMER CONTENT IN LINDANE

Apparatus. The dimensions and general construction of the apparatus used for the routine analysis are illustrated in Figures 7 and 8.

The freezing points were measured with a thermometer constructed to the authors' specifications by Palmer Thermometers, Inc., Cincinnati 12, Ohio. They were 44 cm. long, of Red-Reading Mercury constructed without lens front and calibrated

for 100-mm. immersion. The graduations were in 0.1° intervals from 98° to 120° C. with 25 graduations per inch. When in use the thermometers were calibrated at least once a day at the steam point. The precision of measurement with these thermometers, as determined on six identical samples of known freezing point, was ±0.01° C. The thermometers proved completely satisfactory. The only change contemplated for future thermometers is a 75-mm. immersion line instead of 100 mm.

Analysis of Known Mixtures. In order to prove this method, known mixtures of dry gamma and alpha isomers were analyzed by the freezing point depression, using the value of 0.0148 for the cryoscopic constant.

The known samples were heated to 118° to 120° C. and mixed thoroughly, and the jacket heater and cell pressure were regulated to give a cooling rate of not more than 0.5° C. per minute. The freezing point was taken as the temperature plateau that occurred immediately after recovery from undercooling. The thermometer was read to ±0.01° C. with the aid of a small magnifying glass.

The gamma isomer concentration was calculated from the equation:

$$\text{Log mole \% gamma} = 2 - \frac{0.0148 \times \Delta t}{2.30259} \qquad (4)$$

where Δt = 112.86 − freezing point of sample.

The results obtained on known samples are given in Table III.

The cryoscopic analysis of commercial samples of lindane is complicated by the possible presence of moisture and/or residual solvents. Complete drying can best be effected by dispersing dry nitrogen through the melted material. The possibility of change in composition was investigated by preparing known samples of the dry alpha and gamma isomers, passing dry nitrogen through the melted samples held at 115° C. for varying lengths of time, and then determining the gamma isomer content. These data are summarized in Table IV.

ACKNOWLEDGMENT

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Separation of Chromium from Vanadium

By Extraction of Perchromic Acid with Ethyl Acetate

ROBERT K. BROOKSHIER, *Northwest Electrodevelopment Laboratory, Bureau of Mines, Albany, Ore.,* **AND HARRY FREUND,** *Department of Chemistry, Oregon State College, Corvallis, Ore.*

Research by the U. S. Bureau of Mines on the recovery of vanadium from low grade ferrophosphorus required the determination of small amounts of chromium in vanadic oxide products. The titrimetric method is inaccurate, and Foster's perchromic acid extraction procedure gave erratic results. Study of the variables influencing the extraction led to the following optimum conditions: pH at equilibrium 1.7 ± 0.2, concentration of hydrogen peroxide 0.02 mole per liter, temperature 20° C. or less, and number of extractions 3. Adherence to these conditions, with final estimation of chromium with *s*-diphenylcarbazide, yields a more sensitive and reliable method than any heretofore described.

ACCURACY in the titrimetric determination of chromium in the presence of vanadium (*9*) is difficult to attain when vanadium preponderates. The usual colorimetric determination of chromium (*5*) as chromate or with *s*-diphenylcarbazide necessitates a preliminary separation from vanadium. Foster (*3*) employed ethyl acetate as an immiscible solvent to extract perchromic acid and thereby effected the separation and concentration of small amounts of chromium in vanadium products. Attempts in the laboratory of the U. S. Bureau of Mines to apply the separation to determination of chromium in red-cake vanadic oxide led to erratic results and indicated the need for a systematic study of the factors influencing the separation.

Since 1847, when Barreswil (*1*) first reported the formation of blue perchromic acid by the action of hydrogen peroxide on dichromate, the reaction has been used as a sensitive qualitative test for chromium. The object of later research (*2*, *4*, *7*, *8*) was the study, in a homogeneous system, of catalytic decomposition of hydrogen peroxide by dichromate. The reaction of oxidized compounds with hydrogen peroxide, replacing oxygen atoms with peroxide groups, generally leads to unstable products that lose oxygen very easily. Studies by Spitalsky (*7*, *8*) and Bobtelsky (*2*) confirm the transitory nature of perchromic acid as an intermediate in the catalytic reduction of hydrogen peroxide with dichromate. No attempt was made to stabilize the intermediate perchromic acid, although the free acid was known to influence the reduction, and the presence of an organic solvent in the homogeneous system was shown to have a stabilizing effect (*2*).

REAGENTS AND APPARATUS

Chemically pure reagents were used throughout this work. Absolute ethyl acetate was used and was recovered and purified by distillation after each experiment. The hydrogen peroxide solution was prepared and standardized daily from Baker's C.P. 30% hydrogen peroxide solution. The standard chromium solutions were prepared from accurately weighed amounts of National Bureau of Standards potassium dichromate No. 136. C.P. ammonium metavanadate was used for preparing the standard vanadium solution.

All pH measurements were made with the Beckman Model M pH meter.

The Beckman Model DU spectrophotometer was used.

PROCEDURE FOR CHROMIUM DETERMINATION

In these studies the chromium contents of the ethyl acetate solutions were found by the spectrophotometric diphenylcarbazide method described by Sandell (*5*).

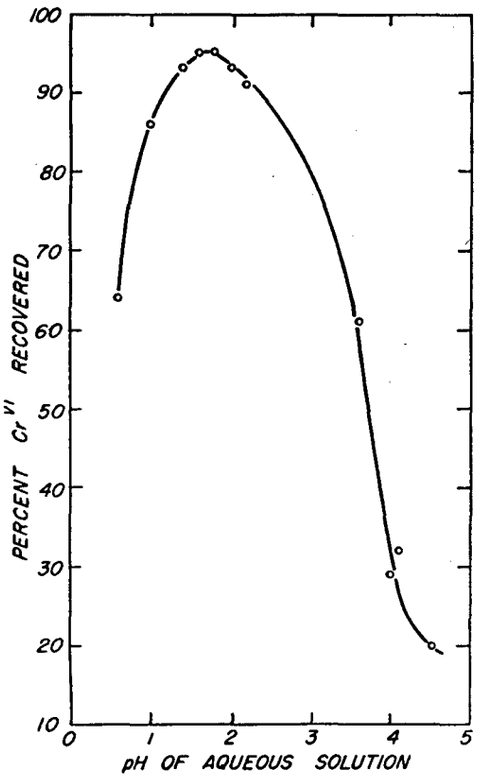

Figure 1. Effect of pH on Recovery of Chromium

Single extraction

One milliliter of 10% potassium hydroxide solution was added to the blue ethyl acetate solution of perchromic acid. This returned the chromium to the aqueous layer and liberated oxygen. The yellow chromate was extracted with water and the solution was boiled to remove peroxide, cooled, and diluted to 50 ml. in a volumetric flask. A suitable aliquot, depending upon the approximate chromium content, was transferred to a 100-ml. volumetric flask, and 50 to 60 ml. of water were added, followed by 1 ml. of 0.25% alcoholic *s*-diphenylcarbazide solution. The whole solution was diluted to 100 ml. and mixed well; after 5 to 8 minutes the absorbancy was measured relative to distilled water at a wave length of 540 mμ and at a slit width of 0.04 mm. The concentration of sexivalent chromium was obtained from a standard linear curve and the per cent recovery of chromium was calculated.

This method is reproducible and far more sensitive than the method based upon the yellow color of chromate ion.

EFFECT OF REACTION VARIABLES

Effect of pH of Aqueous Solution. The effect of pH over the range 0.5 to 4.5 was studied first.

The aqueous solution containing 2.8 mg. of chromium as dichromate, and the desired amount of hydrochloric acid–potassium chloride buffer (*6*) were placed in a 250-ml. separatory funnel and diluted to 50 ml.; 100 ml. of ethyl acetate were added, followed by approximately 1 ml. of 6% hydrogen peroxide solution. The mixture was shaken vigorously for 0.5 minute, the blue perchromic acid passing into the ethyl acetate phase. The aqueous layer was drawn off and its pH measured. Because of the instability of perchromic acid only one extraction was made. After the aqueous layer was drawn off, the chromium in the ethyl acetate was converted to chromate by the addition of potassium hydroxide. The chromate was extracted with water and determined spectrophotometrically with *s*-diphenylcarbazide.

A maximum chromium recovery of 93 to 96% was obtained at pH 1.7 ± 0.2. The data are plotted in Figure 1.

Effect of Sexivalent Chromium Concentration. The above study was repeated for a series of initial weights of chromium varying from 0.4 to 4 mg., a concentration range of 0.000154 to 0.00154 mole per liter. As indicated by Table I, the initial concentration of sexivalent chromium showed no significant effect on its recovery.

Table I. Effect of Sexivalent Chromium Concentration on Recovery

Volume of aqueous layer at equilibrium = 50 ml. pH of aqueous layer at equilibrium = 1.7 ± 0.2

Cr^{VI} Taken, Mg.	Cr^{VI} Found, Mg.	Cr^{VI} Recovery, %
0.400	0.370	93
1.60	1.58	99
2.00	1.87	94
2.80	2.65	95
4.00	3.63	91

Effect of Hydrogen Peroxide Concentration. For this study a fresh solution of hydrogen peroxide was prepared from 30% solution and standardized against potassium permanganate. A known volume of this solution was added to the mixture of ethyl acetate and dichromate, buffered to a pH of 1.7. The perchromic acid was extracted once and chromium(VI) was determined spectrophotometrically as before. As the volume of standard hydrogen peroxide added and the volume of the aqueous solution were known, the hydrogen peroxide concentration was calculated.

These data, listed in Table II, indicate that maximum recovery of sexivalent chromium is obtained when the concentration of hydrogen peroxide in the aqueous solution at the time of extraction is 0.02 mole per liter. Above this concentration the recovery decreases slowly; below this concentration the decrease in recovery is rapid.

Table II. Effect of Hydrogen Peroxide Concentration on Sexivalent Chromium Recovery

Volume of aqueous layer at equilibrium = 50 ml. pH of aqueous solution at equilibrium = 1.7 ± 0.2

Cr^{VI} Taken, Mg.	Cr^{VI} Found, Mg.	Cr^{VI} Recovered, %	Hydrogen Peroxide Concn., Mole/Liter
0.985	0.786	80	0.002
0.985	0.813	83	0.004
0.985	0.848	86	0.012
0.985	0.966	98	0.018
0.985	0.957	97	0.020
2.00	1.94	97	0.020
0.985	0.918	93	0.060
0.985	0.950	97	0.12
0.985	0.892	90	0.19
0.985	0.900	91	0.47

Effect of Successive Extractions. It was expected that repeated extractions would improve the chromium recovery, if the chromium loss was due to partition between the solvents and not to decomposition of the perchromic acid. Three successive extractions were made using 100 ml. of ethyl acetate for the first and 15 ml. for the second and third extractions. The chromium recovery was determined for each extraction separately (Table III). Essentially quantitative recovery of the chromium may thus be attained.

Effect of Temperature. The recovery of sexivalent chromium would be expected to be dependent upon the temperature of the

reaction mixture, as perchromic acid is known to be unstable. The influence of temperature was determined by forming the perchromic acid and extracting at several different temperatures.

For low temperatures the separatory funnel containing the aqueous solution of buffer and dichromate, together with 70 ml. of ethyl acetate, was cooled in the freezing compartment of a refrigerator for 0.5 hour. The temperature of the mixture was measured just before the hydrogen peroxide was added. The aqueous solution was extracted three times, the extracts being combined and the chromium determined in the usual manner. One milliliter of 1 *M* standard hydrogen peroxide solution was sufficient to give the optimum concentration and yet sufficiently small not to effect the temperature of the extraction mixture. For higher temperatures a thermostatically controlled bath was used.

The results of this study showed that at 10° C. or below the blue perchromic acid is very stable. The recovery of sexivalent chromium was consistently near 100%. Above 10° C., however, the results became erratic and the recovery decreased rapidly (Table IV).

Table III. Effect of Successive Extractions on Sexivalent Chromium Recovery

Volume of aqueous solution at equilibrium = 50 ml. Concentration of hydrogen peroxide = 0.02 mole per liter. pH of aqueous solution at equilibrium = 1.7 ± 0.2

Cr^{VI} Taken, Mg.	Cr^{VI} Recovered, Mg				Cr Recovered, %
	1st extract	2nd extract	3rd extract	Total	
0.985	0.966	0.023	0.004	0.993	101
2.00	1.94	0.010	0.004	1.954	98
0.985	0.942	0.020	0.006	0.968	98

Table IV. Effect of Temperature on Sexivalent Chromium Recovery

Concentration of hydrogen peroxide = 0.02 mole per liter. Volume of aqueous solution = 50 ml. pH of aqueous solution = 1.7 ± 0.2. Number of extractions = 3

Cr^{V} Taken, Mg.	Cr^{VI} Found, Mg.	Cr Recovery, %	Temp., ° C.
0.985	0.968	98	4.0
0.985	0.972	99	6.0
2.00	1.95	98	9.0
0.985	0.936	95	19.9
0.985	0.950	96	25.0
0.985	0.587	60	29.3
0.985	0.732	74	35.4

STABILITY OF PERCHROMIC ACID

The stability of the blue perchromic acid was studied in both aqueous and ethyl acetate solutions. A series of aqueous solutions containing the same amount of chromium was adjusted to the pH and temperature specified above. The perchromic acid was formed by the addition of hydrogen peroxide and the aqueous solutions were allowed to stand at 10° C. for periods of from 0 to 15 minutes. The perchromic acid was then extracted with ethyl acetate and the per cent recovery of chromium(VI) was determined.

From Figure 2 it will be seen that blue perchromic acid decomposes rapidly in aqueous solution and that its immediate extraction with ethyl acetate is imperative. The blue substance is very stable in ethyl acetate solution for as long as 30 minutes.

Summary. As a result of the above studies it is possible to establish the following set of optimum conditions.

pH at equilibrium, 1.7 ± 0.2
Concentration of hydrogen peroxide, 0.02 mole per liter
Temperature, 10° C. or less
Number of extractions, 3
Simultaneous extraction with ethyl acetate

Twenty-five milliliters of ethyl acetate followed by two 15-ml. portions were adequate for the extraction of 1 mg. of chromium.

INTERFERENCES

There is no interference from the following elements which might be expected to cause trouble: iron, mercury, vanadium, titanium, nickel, molybdenum. Blanks containing about 50 mg. of each were carried through the procedure and none was extracted by ethyl acetate in any significant amount. The small amount of iron extracted produced an absorbancy in the final solution equivalent to only 0.01 microgram of chromium per milliliter.

Table V. Analysis of Synthetic Samples

Volume of aqueous layer at equilibrium = 50 ml. pH of aqueous layer at equilibrium = 1.7 ± 0.2. Concentration of hydrogen peroxide = 0.02 mole per liter. Number of extractions = 3. Temperature of mixture = 10° C.

V_2O_5 Taken, Mg.	Cr Taken, Mg.	Cr Found, Mg.	% Chromium	
			Found	Actual
100	0.100	0.096	0.096	0.10
100	0.200	0.191	0.19	0.20
100	0.300	0.301	0.30	0.30
100	0.400	0.374	0.37	0.40
100	0.500	0.500	0.50	0.50
100	0.800	0.766	0.76	0.79
100	0.985	1.00	0.99	0.98
100	1.40	1.28	1.26	1.38
100	1.80	1.67	1.64	1.77
100	2.00	1.91	1.87	1.96
100	3.00	2.93	2.84	2.91

PROCEDURE FOR ANALYSIS OF SAMPLES

If the sample is an ore, the usual fusion with sodium peroxide or sodium carbonate and sodium peroxide is recommended. In the case of vanadic oxide products it is sufficient to dissolve the sample in sodium hydroxide and boil the solution with a little sodium peroxide to ensure complete oxidation.

In either case, filter the alkaline solution, containing 0.1 gram of sample, to remove small amounts of iron. Neutralize the filtrate containing chromium(VI) and vanadium(V) with sulfuric acid and evaporate the solution to 15 to 20 ml. If much iron is present, acidify the sample solution with sulfuric acid and oxidize chromium with ammonium persulfate, if necessary. This will prevent loss of chromium during the precipitation of iron. Cool the solution and carefully buffer to a pH of 1.7, using a pH meter.

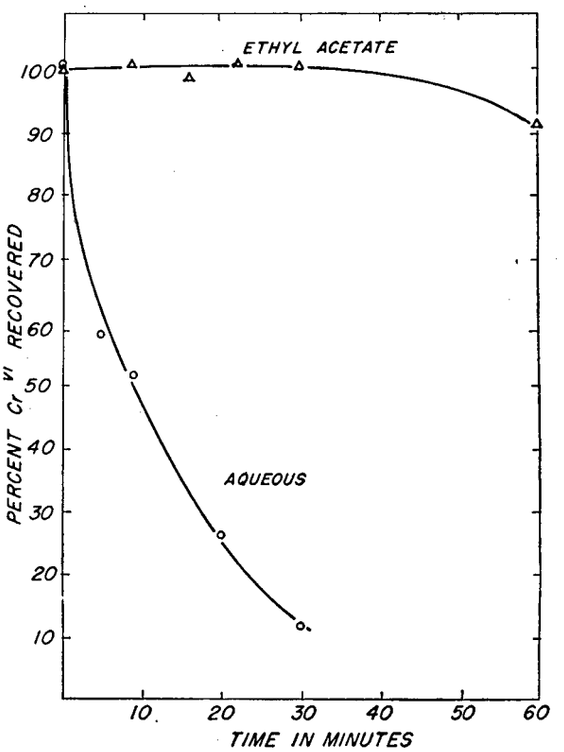

Figure 2. Effect of Solvent on Stability of Perchromic Acid

Transfer this buffered solution to a 250-ml. separatory funnel, dilute to 50 ml., and add 75 ml. of ethyl acetate. Cool the mixture by placing in a refrigerator for 0.5 hour, or if this is impossible, in cold running water. When cool, add 1 ml. of 1 *M* (3.8%) hydrogen peroxide. After shaking the funnel vigorously for 0.5 minute, allow the layers to separate, and draw off the aqueous solution. Repeat the extraction of the aqueous layer at least twice, using 15 ml. of ethyl acetate each time. Combine the ethyl acetate fractions. Add 1 ml. of 10% potassium hydroxide solution to the blue solution of perchromic acid, and shake until the blue color is replaced by yellow. Extract the yellow chromate with water, and boil the solution for 10 minutes. Dilute to 50 ml. and determine the chromium with *s*-diphenylcarbazide.

Analysis of Synthetic Samples. For the analysis of synthetic samples, 100 mg. of vanadic acid as ammonium metavanadate and known weights of sexivalent chromium from 0.1 to 3 mg. were used. The samples were prepared by mixing the proper amounts of standard solutions of vanadate and dichromate.

Eleven such samples were analyzed (Table V). In the range from 0.1 to several per cent, the results are in good agreement with the known values.

Analysis of Standard Sample. National Bureau of Standards ferrovanadium, sample 61A containing 0.68% chromium, was analyzed by this procedure. Parallel analyses were made using 0.100- and 0.250-gram samples and a value of 0.65% chromium was obtained.

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Estimation of Ascorbic Acid in Pharmaceuticals

With Particular Reference to Interfering Substances

D. G. CHAPMAN, ODETTE ROCHON, AND J. A. CAMPBELL

Food and Drug Divisions, Department of National Health and Welfare, Ottawa, Canada

Several methods have been proposed for the estimation of ascorbic acid in the presence of interfering materials such as iron and copper salts, but few data exist on the comparative reliability and limitations of these procedures as applied to pharmaceuticals which may contain these and other interfering substances. Of the eight methods investigated, the procedure of Roe *et al.* was found to be the most reliable under all conditions. The Brown and Adam method was the most satisfactory for routine analysis except in the presence of large amounts of copper. Iron does not interfere in the methods of Gawron and Berg and Frosst, but ascorbic acid may not be stable in the extracting agents used in these methods. The precision of all methods in the absence of interfering substances was found to be satisfactory. Great care must be exercised in the selection of a method for the estimation of ascorbic acid in certain complex pharmaceuticals containing minerals.

AMONG the many methods for the determination of ascorbic acid, those using metaphosphoric acid as the extracting agent and 2,6-dichlorophenolindophenol as the indicator appear to be the most widely used. Ascorbic acid is stable in metaphosphoric acid (*13*) and at the same time its oxidation by copper or enzymes is reduced (*8*). A disadvantage of metaphosphoric acid is that ferrous salts, which are present in many pharmaceutical products, are oxidized quantitatively by indophenol dye. To overcome this objection to metaphosphoric acid, two possibilities are open to the investigator. A reagent may be added to the metaphosphoric acid which will remove the interference caused by iron, or an extracting agent may be sought in which ferrous iron does not react with the dye.

Most of the common acids have been studied (*13*) as possible extracting agents, but ascorbic acid is relatively unstable in all except metaphosphoric and oxalic acids. However, ferrous iron interferes with the estimation of ascorbic acid in both of these, and both metaphosphoric acid (*2*) and oxalic acid (*6*) are themselves relatively unstable.

To eliminate the interference caused by ferrous iron in metaphosphoric acid extracts, Lugg (*11*) has suggested the use of formaldehyde, which at a pH of 3.5 condenses with ascorbic acid but not appreciably with ferrous salts. Because of the large number of titrations which are required for a single determination, this method did not appear to be adaptable to routine analysis and therefore was not studied by the authors. The addition of hydrogen peroxide was first used by Levy (*9*) to eliminate interference by sulfur dioxide. Huelin and Stephens (*7*), however, have reported that for extracts containing ferrous iron, the use of hydrogen peroxide converts a positive error into a negative error of less magnitude. Hydrogen peroxide also promotes a slow reoxidation of the reduced dye and the titration must be completed as soon as possible after adding this reagent. Robinson and Stotz (*14*) have proposed a spectrophotometric method in which certain reducing materials are eliminated by the use of hydrogen peroxide or formaldehyde.

Gawron and Berg (*5*) have suggested using 8% acetic acid to extract ascorbic acid when ferrous iron is present. Brown and Adam (*3*) have reported that a sodium acetate–hydrochloric acid mixture buffered to a pH of 0.65 overcomes the interference due to ferrous iron. Roe *et al.* (*16*) have published a method for the determination of ascorbic acid based on the reaction between dehydroascorbic acid and 2,4-dinitrophenylhydrazine. Roe and Kuether (*15*) have stated that ferrous ions do not interfere in this procedure.

It thus becomes apparent that a variety of methods has been proposed for eliminating or reducing the ferrous iron interference in ascorbic acid assays in foods. However, there is little published information on the relative merits of these methods. Hence this study was undertaken for the purpose of comparing the reliability of eight methods in estimating ascorbic acid, in the presence of both ferrous iron and other substances commonly found in multivitamin pharmaceutical products.

Table I. Methods Examined for Determination of Ascorbic Acid

	Authors	Extracting Agent, % w./v.	Reagent
1.	Roe *et al.* (*16*)	Metaphosphoric acid, 5	2,4-Dinitrophenylhydrazine
2.	Huelin and Stephens using H_2O_2 (*7*)	Metaphosphoric acid, 3	2,6-Dichlorophenolindophenol
3.	U. S. Pharmacopeia XIV (*12*)	Metaphosphoric, 3, plus 8% (v/v) acetic acid	2,6-Dichlorophenolindophenol
4.	Gawron and Berg (*5*)	Acetic acid, 8% (v/v)	2,6-Dichlorophenolindophenol
5.	Watanabe (*17*)	Oxalic acid, 5	2,6-Dichlorophenolindophenol (U.S.P. method)
6.	Frosst (*4*)	Sulfuric acid, 2.5	Iodine 0.005 *N*
7.	Brown and Adam (*3*)	Sodium acetate-hydrochloric acid buffered to pH 0.65	2,6-Dichlorophenolindophenol
8.	Robinson and Stotz using H_2O_2 (*14*)	Metaphosphoric acid, 6, buffered to pH 3.8	2,6-Dichlorophenolindophenol

EXPERIMENTAL

Methods Studied. Eight methods were chosen for study as representative of the available chemical procedures for the routine analysis of ascorbic acid (Table I), and were followed exactly as stated by the authors. In addition to the six methods which were taken from the literature, two other extracting agents, 5% oxalic acid and 2.5% sulfuric acid, were used.

Watanabe (*17*) first recommended the use of oxalic acid as the best extractant for ascorbic acid, although he later used a mixture of metaphosphoric and oxalic acids (*18*). For the present study the authors used a 5% oxalic acid solution and titrated against indophenol dye prepared according to the U. S. Pharmacopeia method (*12*).

Because stability of ascorbic acid in sulfuric acid is very poor (*7*), its use as an extractant for ascorbic acid is not recommended. Nevertheless, it has been found useful by Frosst (*4*) for the estimation of ascorbic acid in the presence of iron. The titrations were carried out one minute after adding the ascorbic acid to the sulfuric acid solution.

As King (*8*) has found that for most investigations direct visual titration of ascorbic acid with indophenol dye gives reasonably accurate and satisfactory results, and this type of method required a minimum of equipment, six of the methods chosen for study employed a titrimetric technique.

Added Substances. The possible interference which might be caused by ferrous sulfate, cuprous chloride, cupric sulfate, ferrous sulfate plus cupric sulfate, liver fraction (liver fraction L obtained from the Nutritional Biochemical Corp., Cleveland, Ohio), and cod liver oil in the recovery of ascorbic acid was investigated. To 100 mg. of ascorbic acid was added 1 gram of one of the interfering materials (1 ml. in the case of cod liver oil) and both were quantitatively transferred to a 100-ml. volumetric flask and made to volume with the respective extracting agents. This ratio of 10 to 1 for ferrous sulfate to ascorbic acid is similar to that which is encountered in some pharmaceutical products. The same ratio was used for the copper salts, although smaller amounts are usually present in pharmaceuticals.

The values reported for the recovery of the ascorbic acid are the average of at least two determinations when 1 ml. of the solution was taken for analysis. In the case of pure ascorbic acid and ascorbic acid plus ferrous iron, five determinations were made.

Pharmaceuticals Examined. Ten multivitamin pharmaceutical products, five of which contained ferrous iron, were assayed for ascorbic acid by each of the eight methods. At least five tablets or capsules were taken for analysis and made to volume with the various extracting agents, so that each milliliter of final dilution contained approximately 1 mg. of ascorbic acid. The tablets were finely ground and quantitatively transferred to a volumetric flask, using the respective extracting agents. The determinations were carried out in duplicate and the average value was reported.

RESULTS AND DISCUSSION

Effect of Added Substances. Table II shows the per cent recoveries of ascorbic acid, in the presence of the various added substances by each of the eight methods under investigation. Cod liver oil caused no appreciable interference in any of the methods examined. Liver fraction, on the other hand, gave a slightly positive error. In the titrimetric methods this was probably due to an obscuring of the end point. Table III shows the mean and the standard deviation of five determinations of ascorbic acid alone and in the presence of ferrous iron by each of the eight methods.

Roe *et al.* (*16*). The procedure for total ascorbic acid was used. This would give a measure of reduced ascorbic acid, dehydroascorbic acid, and diketo-L-gulonic acid. Although the diketo-L-gulonic acid is biologically inactive, it is highly improbable that there would be any appreciable amounts of this substance in pharmaceutical products. Roe and Kuether (*15*) have stated that ferrous or stannous ions cause no interference. The authors' results indicate that none of the added materials cause interference in the recovery of ascorbic acid. The precision of the method as shown by the small standard deviations is good.

Huelin and Stephens (*7*). The hydrogen peroxide modification of this procedure gave extremely low recoveries in the presence of ferrous sulfate. Unsatisfactory recoveries were also obtained when copper salts were present.

United States Pharmacopeia (*12*). This method gave an extremely high recovery of ascorbic acid when ferrous iron was present. This was not unexpected, because the A.O.A.C. method (*1*), which is essentially that of the U. S. Pharmacopeia, is not recommended for use in the presence of ferrous iron. Cuprous chloride appeared to result in a low recovery, whereas cupric sulfate caused no interference.

Gawron and Berg (*5*). Ferrous iron caused no appreciable interference. However, the presence of cuprous chloride, cupric sulfate, and ferrous sulfate plus cupric sulfate resulted in low recoveries of the ascorbic acid. Gawron and Berg (*5*) did not

Table II. Effect of Certain Added Substances on Recovery of Ascorbic Acid as Determined by Eight Methods

(Recoveries in per cent)

		Substance Added[a]					
	Method of Analysis	Ferrous sulfate	Cuprous chloride	Cupric sulfate	Ferrous sulfate plus cupric sulfate	Liver fraction	Cod liver oil
1.	Roe *et al.* (*16*)	100.3	101.2	101.2	101.2	100.0	100.0
2.	Huelin and Stephens using H_2O_2 (*7*)	9.2	71.9	92.7	9.2	105.5	98.2
3.	U.S.P. XIV (*12*)	378.8	89.5	98.5	248.2	106.9	99.2
4.	Gawron and Berg (*5*)	97.9	75.5	65.7	38.0	101.7	97.3
5.	Watanabe (*17*) using 5% oxalic acid	146.4	97.1	100.6	234.7	108.2	99.4
6.	Frosst using 2.5% H_2SO_4 (*4*)	99.7	99.5	100.6	88.8	112.4	98.5
7.	Brown and Adam (*3*)	98.7	211.5	43.2	37.3	105.6	98.9
8.	Robinson and Stotz using H_2O_2 (*14*)	82.8	87.4	87.5	76.5	107.7	102.5

[a] Added at rate of 1 gram to 100 mg. of ascorbic acid.

Table III. Recoveries of Ascorbic Acid Alone and in Presence of Ferrous Iron

		Recoveries, %[a]	
	Method	Ascorbic acid alone	Ascorbic acid plus Fe[b]
1.	Roe *et al.* (*16*)	100.3 ± 0.5	100.3 ± 0.9
2.	Huelin and Stephens using H_2O_2 (*7*)	100.3 ± 1.0	9.2 ± 1.9
3.	U. S. P. XIV (*12*)	100.3 ± 0.4	378.8 ± 13.2
4.	Gawron and Berg (*5*)	100.2 ± 0.6	97.9 ± 0.5
5.	Watanabe (*17*) using 5% oxalic acid	101.0 ± 0.3	146.4 ± 24.3
6.	Frosst using 2.5% H_2SO_4 (*4*)	99.9 ± 0.8	99.7 ± 1.3
7.	Brown and Adam (*3*)	99.8 ± 0.7	98.7 ± 0.5
8.	Robinson and Stotz using H_2O_2 (*14*)	100.9 ± 1.2	82.8 ± 5.1

[a] With standard deviations of five determinations.
[b] Ferrous iron added at rate of 1 gram to 100 mg. of ascorbic acid.

Table IV. Determination of Ascorbic Acid in Pharmaceutical Products[a]

Product	A	B	C	D	E	F	G	H	I	J
Ferrous iron label claim, mg.	None	None	None	None	None	130	162	110	65	300
Ascorbic acid, mg.										
Label claim	17.5	50	30	50	10	50	15	25	40	25
Found by method of Roe *et al.* (*16*)	17.5	51.0	29.4	50.0	7.0	59.0	22.0	28.0	55.0	23.3
Ascorbic acid, as % of Roe's method, found by methods of										
Huelin and Stephens using H_2O_2 (*7*)	98.3	101.2	99.3	102.0	98.6	61.0	27.8	47.5	65.5	22.7
U.S.P. XIV (*12*)	100.6	101.6	100.7	99.8	102.9	190.2	440.5	211.1	298.2	300.4
Gawron and Berg (*5*)	97.7	101.8	99.3	102.0	100.0	103.7	97.3	93.6	98.2	97.9
Watanabe (*17*) using 5% oxalic acid	100.0	99.4	100.3	100.0	100.0	118.5	124.5	103.6	104.7	135.2
Frosst using 2.5% H_2SO_4 (*4*)	97.7	103.3	101.4	102.8	101.4	104.1	104.1	110.0	98.5	106.4
Brown and Adam (*3*)	98.3	98.2	100.7	98.0	97.1	101.2	100.9	100.4	99.6	100.9
Robinson and Stotz using H_2O_2 (*14*)	98.9	100.0	98.0	102.0	94.3	102.5	88.6	96.4	99.1	93.1

[a] Results expressed in mg. for Roe's method and as percentage of that found by Roe's method for each of other procedures.

study the possible interference due to copper salts, nor did they report the stability of ascorbic acid in acetic acid. However, Ponting (*13*) has shown that ascorbic acid is not stable in the presence of acetic acid.

Watanabe (*17*). Ferrous sulfate and ferrous sulfate plus cupric sulfate resulted in high and variable recoveries of ascorbic acid in oxalic acid. This is consistent with the findings of Lorenz and Arnold (*10*), who reported that ferrous salts titrate with indophenol dye in solutions of oxalic acid. The chief advantage of oxalic acid is its stabilizing action on ascorbic acid even in the presence of copper (*13*). Under the conditions of the authors' experiment the copper salts caused no interference in the recovery of ascorbic acid.

Frosst (*4*). When this method was used, ferrous sulfate, cuprous chloride, and cupric sulfate caused no interference. Ferrous sulfate plus cupric sulfate, however, resulted in a low recovery of ascorbic acid. It is evident from Table III that this procedure can give reproducible results if sufficient care is taken to allow for the instability of ascorbic acid in sulfuric acid.

Brown and Adam (*3*). Unfortunately, these authors do not indicate how the sodium acetate–hydrochloric acid solution buffered to pH 0.65 was prepared. The present authors prepared a 1 *N* hydrochloric acid solution and added 1 *N* sodium acetate to this until a pH of 0.65, as indicated on the Beckman pH meter, was obtained. Brown and Adam have shown that ferrous iron does not cause interference with the titration of ascorbic acid when using their extracting medium. The data reported here would confirm this. When cuprous chloride was added to ascorbic acid, it introduced a large positive error. Cupric sulfate, on the other hand, resulted in a very low recovery of the ascorbic acid. Although this is contrary to the findings of Brown and Adam, who report that copper sulfate does not interfere, those authors were using a solution containing 2 mg. of ascorbic acid and 5 mg. of copper sulfate in 100 ml., whereas the solution used by the present authors contained 100 mg. of ascorbic acid and 1000 mg. of copper sulfate in 100 ml. Using the amounts suggested by Brown and Adam, no interference in the recovery of ascorbic acid was encountered. Both ferrous sulfate and cupric sulfate together gave a low recovery. The precision of this method was good, as can be seen in Table III.

Robinson and Stotz using hydrogen peroxide (*14*). Under the conditions of this experiment, ferrous sulfate, cuprous chloride, cupric sulfate, and cupric sulfate plus ferrous sulfate all interfered with the recovery of ascorbic acid. If, however, an amount of ferrous iron similar to that suggested by Robinson and Stotz (molar ratio of ferrous ion to ascorbic acid of 1 to 1) was used, 100% recovery of ascorbic acid was obtained. The standard deviation for pure ascorbic acid, when using this method, was 1.2.

Assay of Ascorbic Acid in Pharmaceuticals. The results of the ascorbic acid determinations on the pharmaceutical products are given in Table IV. As the procedure of Roe *et al.* (*16*) was found to be the most reliable in the presence of possible interfering materials, it was used as a standard and the values obtained by the other methods are expressed as a percentage of it.

All the methods gave very similar and satisfactory results for products that contained no ferrous sulfate. Product E appeared to be below the labeled potency of 10 mg. per tablet, as no method gave a value higher than 7.2 mg.

If ferrous iron was present, the methods gave varying values for the ascorbic acid content. The hydrogen peroxide modification of the method of Huelin and Stephens (*7*) gave consistently low recoveries. Generally speaking, the more iron that was present, the lower was the recovery of the ascorbic acid. As was to be expected, the U.S.P. method (*12*) gave values far in excess of that found by Roe's method. The procedure of Gawron and Berg (*5*) gave results which with but one exception were in good agreement with those given by Roe's method. When using 5% oxalic acid the recoveries of ascorbic acid were high. This is consistent with the values reported in Table II, which show that iron introduces a positive error in the presence of oxalic acid.

The 2.5% sulfuric acid solution gave slightly high recoveries in the presence of ferrous iron. This may be explained by the fact that many pharmaceuticals impart considerable color to the extracting solutions, making the detection of the iodine-starch end point very difficult to observe. This difficulty had the effect of lowering the precision of the method. Furthermore, ascorbic acid is unstable in sulfuric acid (*7*). The method of Brown and Adam (*3*) gave values that were in excellent agreement with those found by Roe's method. While copper may interfere in the Brown and Adam method (Table II), product J was the only one containing copper (1.6 mg. per tablet), and this amount caused no discrepancy. The hydrogen peroxide modification of the method of Robinson and Stotz (*14*) gave slightly low recoveries for three of the five products that contained iron.

CONCLUSION

In the absence of ferrous iron, all methods investigated gave satisfactory results for the determination of ascorbic acid in pharmaceuticals. In the presence of ferrous iron the method of Brown and Adam proved to be most suitable for routine analyses. However, if both ferrous iron and copper were present, the method of Roe *et al.* was most reliable.

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Estimation of Antioxidants in Lard and Shortening

J. H. MAHON AND R. A. CHAPMAN

Food and Drugs Laboratory, Department of National Health and Welfare, Ottawa, Canada

Because mixtures of antioxidants are now widely used in the stabilization of lard and shortening, methods for the determination of combinations of antioxidants are required. Procedures have been developed for the extraction and colorimetric determination of propyl gallate, butylated hydroxyanisole, nordihydroguaiaretic acid, and tocopherol in lard and shortening. The procedure permits the determination of all combinations of these four antioxidants except when propyl gallate and nordihydroguaiaretic acid are present in the same sample. The lower limits of the determinations, with a high degree of accuracy, are propyl gallate 0.003%, butylated hydroxyanisole 0.005%, nordihydroguaiaretic acid 0.005%, and tocopherol 0.015%. However, satisfactory results have been obtained at much lower levels. Recoveries ranged from 96.4% for nordihydroguaiaretic acid in combination with butylated hydroxyanisole, to 98.4% for nordihydroguaiaretic acid alone. These procedures permit accurate determination of combinations of antioxidants in lard and shortening, as well as study of the relative rates of destruction of individual antioxidants in mixtures in lard and shortening.

PROPYL gallate (PG), butylated hydroxyanisole (BHA), nordihydroguaiaretic acid (NDGA), and tocopherol are considered to be the most important antioxidants used in food products in Canada and the United States. Methods are described in the literature for the determination of these individual antioxidants, but there are no procedures for the quantitative determination of mixtures. As the present trend is to employ combinations of antioxidants in lard and shortening, there is a definite need for a procedure by means of which mixtures of antioxidants may be determined in these products. The following methods are capable of determining mixtures of antioxidants present in lard and shortening in the concentrations usually employed.

Mitchell (*7*) reported that gallic acid and other trihydroxyphenols react with ferrous tartrate to form an intense purple color which he believed to be specific for the pyrogallic grouping. Later, Glasstone (*2*) investigated the influence of pH on this reaction with gallic acid and recommended the use of an ammonium acetate buffer of pH 7.0. Lundberg and Halvorson (*5*) stated that ferrous tartrate gives a colored reaction product with most, if not all, polyphenols but that the products are insoluble in many cases and, therefore, cannot be estimated colorimetrically. Mattil and Filer (*6*), using Glasstone's modification, found that the soluble complex formed with gallic acid exhibited a maximum absorption at 540 mμ and obeyed Beer's law over the range of 0.2 to 1.0 mg. per 100 ml. of solution. Propyl gallate has been found to react with ferrous tartrate in an analogous manner.

The 1,1′-bipyridine reagent has been employed by Hill (*3*) for the colorimetric estimation of iron in biological material. Emmerie and Engel (*1*) later used ferric chloride plus 1,1′-bipyridine for the colorimetric estimation of tocopherol. The literature contains many applications of this reaction to the determination of reducing substances, including propyl gallate, nordihydroguaiaretic acid, and butylated hydroxyanisole (*4*).

Preliminary experiments indicated that the ferrous tartrate reagent for propyl gallate and the ferric chloride plus 1,1′-bipyrine reagent for butylated hydroxyanisole, nordihydroguaiaretic acid, and tocopherol were satisfactory; therefore, these reagents were studied more intensively.

EXTRACTION OF ANTIOXIDANTS FROM LARD AND SHORTENING

It appeared possible that propyl gallate, butylated hydroxyanisole, nordihydroguaiaretic acid, and tocopherol could be extracted individually or in groups on the basis of their differential solubilities in water, ethyl alcohol, and a petroleum ether solution of the fat. Preliminary experiments indicated that propyl gallate and a portion of the nordihydroguaiaretic acid could be removed from a solution of lard or shortening in petroleum ether by aqueous extraction, whereas neither butylated hydroxyanisole nor tocopherol was removed by this treatment.

Lundberg *et al.* (*5*) have reported that the quantitative extraction of a number of antioxidants from fats, with the exception of tocopherol, could be achieved using 80% ethyl alcohol. In this investigation it was found that repeated extraction of a petroleum ether solution of the fat with 80% ethyl alcohol quantitatively removed propyl gallate, butylated hydroxyanisole, and nordihydroguaiaretic acid and also a portion of the tocopherol. However, by reducing the ethyl alcohol content to 72% it was possible to extract the propyl gallate, butylated hydroxyanisole, and nordihydroguaiaretic acid and leave 98.5% of the tocopherol in the petroleum ether solution of the fat. When alcohol concentrations lower than 72% were employed, the extraction of butylated hydroxyanisole was incomplete and therefore 72% ethyl alcohol was chosen as the extracting medium.

The effect of ethyl alcohol concentration on the extraction of butylated hydroxyanisole and tocopherol from a fat solution is shown in Figure 1. Owing to the similar solubilities of propyl gallate and nordihydroguaiaretic acid, these antioxidants cannot be separated completely when both are present in the same sample. However, as combinations of propyl gallate and nordihydroguaiaretic acid are not employed as antioxidants in foods in

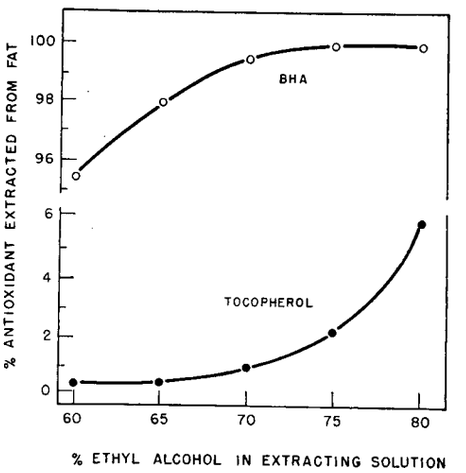

Figure 1. Effect of Alcohol Concentration on Extraction of Butylated Hydroxyanisole and Tocopherol from Petroleum Ether Solution of a Fat

BHA. 2 mg. of BHA, 10 grams of fat in 40 ml. of petroleum ether
Tocopherol. 10 mg. of tocopherol, 10 grams of fat in 40 ml. of petroleum ether
Four 3-minute extractions with 25-ml. aliquots of alcoholic solutions

either Canada or the United States, it is possible, by applying qualitative tests, outlined below, to ascertain the presence or absence of propyl gallate. If propyl gallate is present, it can be extracted into the aqueous phase and the absence of nordihydroguaiaretic acid assumed. If propyl gallate is absent, the aqueous extraction is omitted and nordihydroguaiaretic acid and/or butylated hydroxyanisole is extracted with 72% alcohol. The aqueous and alcoholic extraction procedures can be used consecutively for the extraction of propyl gallate and butylated hydroxyanisole from a petroleum ether solution of a fat. In all cases the tocopherol remains in the fat solution.

DEVELOPMENT OF REAGENTS

Propyl Gallate. The ferrous tartrate reagent as modified by Glasstone (*2*) was used for the colorimetric analysis of propyl gallate. This reagent was found to be specific for propyl gallate among the four antioxidants under consideration. Although a high concentration of nordihydroguaiaretic acid does produce a blue precipitate with ferrous tartrate, this precipitate is insoluble and can be removed by centrifuging, thus producing no error in the colorimetric determination of propyl gallate. Although the ferrous tartrate reagent is less sensitive than the ferric chloride plus 1,1′-bipyridine reagent, the former was adopted because of its specificity, rapidity of color formation, and stability of the color. The ferrous tartrate–propyl gallate colored complex exhibits a maximum absorption at 540 mμ, and obeys Beer's law over the range of 60 to 360 micrograms of propyl gallate per 25 ml. of solution. The rate of color development is shown in Figure 2.

Butylated Hydroxyanisole and Nordihydroguaiaretic Acid. Maximum color formation was obtained when 2 ml. of 0.2% ferric chloride ($FeCl_3.6H_2O$) reagent and 2 ml. of 0.2% 1,1′-bipyridine reagent were added to not more than 50 micrograms of the foregoing antioxidants contained in 8 ml. of 82% ethyl alcohol (butylated hydroxyanisole extract in 5 ml. of 72% ethyl alcohol plus 3 ml. of 100% ethyl alcohol). With this reagent combination effective maxima of color formation were obtained after 30 minutes for commercial butylated hydroxyanisole preparations and after 3 minutes for nordihydroguaiaretic acid. Commercial preparations of butylated hydroxyanisole available for this study were mixtures of the isomers, 2-*tert*-butyl-4-hydroxyanisole and 3-*tert*-butyl-4-hydroxyanisole. It was found that the 3-*tert*-butyl-4-hydroxyanisole reacts more rapidly with ferric chloride plus α,α'-bipyridine than 2-*tert*-butyl-4-hydroxyanisole, but that 2-*tert*-butyl-4-hydroxyanisole produces more color per unit weight than 3-*tert*-butyl-4-hydroxyanisole. Results indicate that maximum color formation is obtained in 15 minutes with 3-*tert*-butyl-4-hydroxyanisole, in 45 minutes with 2-*tert*-butyl-4-hydroxyanisole, and in 30 minutes with three commercial butylated hydroxyanisole preparations. Over the range of 10 to 50 micrograms of butylated hydroxyanisole in 12 ml. of solution, the color formed obeyed Beer's law. The color formed with nordihydroguaiaretic acid also obeyed Beer's law over the same concentration range, when the absorbancy was measured after 3 minutes. Table I shows the absorbancy per microgram of various butylated hydroxyanisole preparations and nordihydroguaiaretic acid on reaction with the ferric chloride plus 1,1′-bipyridine reagent.

For all subsequent work commercial butylated hydroxyanisole preparation No. 1 was used as a reference standard and 30 minutes were allowed for color development.

Table I. Absorbancy per Unit Weight of Various Butylated Hydroxyanisole Preparations and Nordihydroguaiaretic Acid on Reaction with Ferric Chloride plus 1,1′-Bipyridine Reagent

Antioxidant	Reaction Time, Minutes	Absorbancy/γ at 515 mμ
Pure 3-BHA	30	0.0154
Commercial BHA		
No. 1	30	0.0155
No. 2	30	0.0156
No. 3	30	0.0156
Pure 2-BHA	30	0.0170
NDGA	3	0.0155
NDGA	30	0.0161

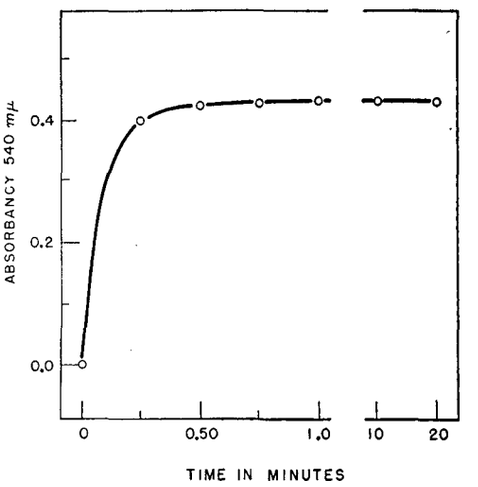

Figure 2. Rate of Reaction of Propyl Gallate with Ferrous Tartrate Reagent

Tocopherol. For the determination of tocopherol, 2 ml. of 0.2% ferric chloride plus 2 ml. of 0.5% 1,1′-bipyridine were found to give the most satisfactory results under the conditions employed. The exact procedure is given in the section outlining the method for the determination of tocopherol.

REAGENTS

Petroleum Ether. Mix 1 volume of 30° to 60° C. petroleum ether with 3 volumes of 60° to 100° C. petroleum ether (Skellysolve H) and shake the mixture with one tenth its volume of concentrated sulfuric acid for 5 minutes. Run off the yellowish acid layer and wash the petroleum ether repeatedly with water and then with 1% potassium hydroxide solution until free of acid. Distill the petroleum ether, using an all-glass apparatus.

Ethyl Alcohol. To absolute alcohol add approximately 0.1% of potassium hydroxide and 0.1% of potassium permanganate. Distill in all-glass apparatus. The distillate is used for the 100% alcohol and is diluted with water to contain 86 and 72% alcohol by volume.

Ferrous Tartrate, 0.1% hydrated ferrous sulfate ($FeSO_4.7H_2O$) and 0.5% of Rochelle salt in distilled water, freshly prepared.

Ammonium Acetate, 1.25, 1.67, and 10.0% solutions. A solution is also required containing 1.67% of ammonium acetate in 5% aqueous ethyl alcohol.

Ferric Chloride, 0.2% solution of hydrated ferric chloride ($FeCl_3.6H_2O$) in purified absolute ethyl alcohol. This reagent must be fresh to avoid highly colored blanks.

1,1′-Bipyridine, 0.2% and 0.5% solutions in purified absolute ethyl alcohol.

Borax, 2.0% aqueous solution of borax ($Na_2B_4O_7.10H_2O$).

QUALITATIVE TESTS

Place approximately 10 grams of the fat in a separatory funnel and dissolve in 50 ml. of petroleum ether. Extract the fat solution by shaking 3 minutes with 20 ml. of 72% ethyl alcohol.

Propyl Gallate. To 5 ml. of the alcoholic extract in a test tube add 10 drops of concentrated ammonium hydroxide. The appearance of a rose color indicates the presence of propyl gallate.

Butylated Hydroxyanisole. To 5 ml. of the extract add 1 ml. of 2% aqueous borax reagent and a few tiny crystals of 2,6-dichloroquinonechlorimide. The appearance of a blue color indicates the presence of butylated hydroxyanisole. If an excessive amount of 2,6-dichloroquinonechlorimide is added, the color formed may be greenish blue. This test will detect less than 1 p.p.m. of butylated hydroxyanisole under optimum conditions. Gum guaiacum also produces a blue color.

Nordihydroguaiaretic Acid. If qualitative tests indicate the absence of butylated hydroxyanisole and propyl gallate, add 2 ml. of ferric chloride reagent and 2 ml. of 0.2% 1,1′-bipyridine reagent to a 5-ml. aliquot of the extract. The appearance of a red color is indicative of nordihydroguaiaretic acid.

Nordihydroguaiaretic Acid and Butylated Hydroxyanisole. If the butylated hydroxyanisole test is positive, it is necessary to test for the presence of nordihydroguaiaretic acid as follows:

To a suitable aliquot of the extract, diluted to 5 ml. with 72% of ethyl alcohol, add 3 ml. of absolute ethyl alcohol, followed by 2 ml. of the ferric chloride reagent and 2 ml. of 0.2% 1,1′-bipyridine reagent. Determine the ratio of the optical densities measured at 1 and 10 minutes after the addition of the reagents. The aliquot should be chosen so that the absorbancy measured at 10 minutes does not exceed 0.8 as measured by an Evelyn photoelectric colorimeter fitted with a 515 mμ filter. Compare the results obtained with those given in Table II and Figure 3.

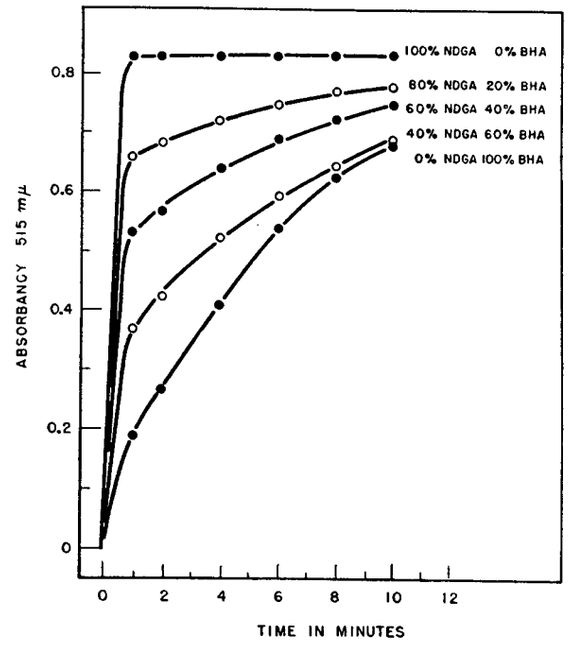

Figure 3. Effect of Ratio of Nordihydroguaiaretic Acid to Butylated Hydroxyanisole on Rate of Color Formation with Ferric Chloride plus 1,1′-Bipyridine Reagents

Tocopherol. No attempt was made to develop a qualitative test for tocopherol, as it was found in all lards and shortenings examined.

QUANTITATIVE PROCEDURE

Dissolve 50 grams of the fat in purified petroleum ether and make up to 250 ml. Gentle warming may be necessary to obtain complete solution.

Propyl Gallate. Pipet 50 ml. of the fat solution into a 250-ml. separatory funnel. Extract the fat solution with three 20-ml. volumes of aqueous 1.67% ammonium acetate solution by continuously inverting the separatory funnel for 2.5 minutes, and finally extract the solution for a few seconds with 15 ml. of water. Allow time after each extraction for complete separation of the phases before running off the aqueous layer.

Dilute the combined extracts to 80 ml. with water. This solution now contains 1.25% of ammonium acetate. Pipet three different aliquots of the extract not exceeding 20 ml. into 40-mm. rectangular Coleman absorption cells and dilute all aliquots to 20 ml. with 1.25% ammonium acetate solution. Add 4 ml. of water and 1 ml. of ferrous tartrate reagent to each cell. Stir the contents and after 3 minutes measure the absorbancy at 540 mμ in a Coleman Universal spectrophotometer. All absorbancies should be measured relative to a blank containing 20 ml. of 1.25% ammonium acetate solution, 4 ml. of water, and 1 ml. of ferrous tartrate reagent.

Table II. Ratio of Absorbancies Measured at 1 and 10 Minutes as Related to Proportions of Butylated Hydroxyanisole and Nordihydroguaiaretic Acid

% NDGA	% BHA	Ratio of Absorbancies at 1 Min./10 Min.
100	0	0.99
80	20	0.85
60	40	0.71
40	60	0.54
20	80	0.35
0	100	0.27

Place suitable aliquots of a standard aqueous propyl gallate solution (20 micrograms per ml.) in 40-mm. absorption cells, add 2.5 ml. of 10% ammonium acetate solution, dilute to 24 ml. with water, and add 1 ml. of ferrous tartrate reagent. Measure the absorbancy as previously described and plot a reference curve over the range of 20 to 400 micrograms of propyl gallate.

Under the above conditions, the observed absorbancy divided by a k value of 0.00223 gives the concentration of propyl gallate in micrograms per aliquot used.

In the case of certain shortenings, a strong tendency to emulsify was noted during the aqueous extraction of propyl gallate. To prevent this emulsification, 2 ml. of 1-octanol are added to the aliquot of fat solution before the extraction is begun. A 1.67% ammonium acetate solution in 5% ethyl alcohol should be used for the extraction in place of the aqueous ammonium acetate solution. This procedure need be used only when the normal method fails. The use of octanol and 1.67% ammonium acetate in 5% aqueous ethyl alcohol solution yielded results of the same magnitude and accuracy as obtained with the original method.

Butylated Hydroxyanisole. Propyl gallate, if present, must be removed by the foregoing method before proceeding with the extraction of butylated hydroxyanisole.

Extract butylated hydroxyanisole from the remaining fat solution with three 25-ml. aliquots of 72% ethyl alcohol by continuously inverting the separatory funnel for 3 minutes. Carry out a further extraction for 1 minute with 60 ml. of 72% ethyl alcohol. Allow time after each extraction for complete separation of the phases. Combine the four extracts and make up to 150 or 200 ml. with 72% ethyl alcohol, depending upon the butylated hydroxyanisole concentration.

Place 3 different aliquots of the butylated hydroxyanisole extract, varying from 1 to 5 ml., in 30-ml. Erlenmeyer flasks provided with glass stoppers. These flasks must be rendered impervious to light with black tape, owing to the instability of the reagents. Dilute the aliquots in all flasks to 5 ml. with 72% ethyl alcohol. Add 3 ml. of 100% ethyl alcohol, 2 ml. of 0.2% ferric chloride reagent, and 2 ml. of 0.2% 1,1′-bipyridine reagent to each flask. Immediately stopper the flasks and gently swirl the contents. Thirty minutes after adding the ferric chloride reagent, transfer the colored solution to an 18-mm. colorimeter tube and measure the absorbancy with an Evelyn photoelectric colorimeter using a 515 mμ filter. All measurements are made relative to a blank containing 5 ml. of 72% ethyl alcohol, 3 ml. of 100% ethyl alcohol, 2 ml. of 0.2% ferric chloride reagent, and 2 ml. of 0.2% 1,1′-bipyridine reagent.

The reference curve was prepared over the range of 10 to 50 micrograms of butylated hydroxyanisole. The concentration of butylated hydroxyanisole in micrograms per aliquot used was obtained by dividing the observed absorbancy by a k value of 0.0155. It is necessary to use freshly prepared ferric chloride reagent in order to avoid highly colored blanks. The reference curve should be checked at frequent intervals.

Nordihydroguaiaretic Acid. The extraction procedure for nordihydroguaiaretic acid is identical with that given for butylated hydroxyanisole. However, nordihydroguaiaretic acid cannot be determined after the extraction of the propyl gallate with aqueous 1.67% ammonium acetate solution, because this extraction also removes a considerable portion of the nordihydroguaiaretic acid. The analytical procedure for nordihydroguaiaretic acid is identical to that given for butylated hydroxyanisole, except that the absorbancy is measured after 3 minutes. The concen-

tration of nordihydroguaiaretic acid in micrograms per aliquot is obtained by dividing the observed absorbancy by a k value of 0.0155.

Combinations of Butylated Hydroxyanisole and Nordihydroguaiaretic Acid. If both nordihydroguaiaretic acid and butylated hydroxyanisole are present in the same fat sample, they will both be extracted with 72% ethyl alcohol. However, it is possible to determine the amounts of each of these antioxidants, as they react at different rates with ferric chloride plus 1,1′-bipyridine.

The reagents and method of analysis are identical to those described for the individual determination of butylated hydroxyanisole and nordihydroguaiaretic acid. However, when both compounds are present it is necessary to set up a duplicate series of tubes and to determine the absorbancy of one series of tubes after 1 minute and the absorbancy of the other series after 30 minutes. The absorbancy measured after 1 minute is due to the reaction of 90% of the nordihydroguaiaretic acid and 10% of the butylated hydroxyanisole. Similarly, the absorbancy measured after 30 minutes is due to the reaction of 100% of the nordihydroguaiaretic acid and 100% of the butylated hydroxyanisole. Therefore, it was possible to derive the following simultaneous equations:

$$L \text{ at 1 minute} = 0.1\,B + 0.9\,N$$

$$L \text{ at 30 minutes} = 1.0\,B + 1.0\,N$$

where L is observed absorbancy, B is absorbancy due to butylated hydroxyanisole, and N is absorbancy due to nordihydroguaiaretic acid.

By means of the foregoing equations it is possible to calculate the respective absorbancies due to nordihydroguaiaretic acid and butylated hydroxyanisole. The value of N divided by a constant of 0.0161 (see Table I) and the value of B divided by 0.0155 were found to give the concentrations of nordihydroguaiaretic acid and butylated hydroxyanisole, respectively, in micrograms per aliquot.

Tocopherol. The fat solution in petroleum ether, from which the propyl gallate, butylated hydroxyanisole, and/or nordihydroguaiaretic acid has been extracted, is used for the determination of tocopherol. Owing to the depressing effect of fat on the color produced per unit weight of tocopherol in the presence of ferric chloride plus 1,1′-bipyridine, it is necessary to use an internal tocopherol standard.

Transfer the fat solution after extraction of propyl gallate, butylated hydroxyanisole, and/or nordihydroguaiaretic acid to a 250-ml. volumetric flask and wash the separatory funnel several times with petroleum ether. Combine the washings and dilute to volume with petroleum ether. If qualitative tests have shown that the fat does not contain propyl gallate, butylated hydroxyanisole, or nordihydroguaiaretic acid, a 4% solution of the fat in petroleum ether can be employed without any previous extraction. Place three duplicate aliquots of the fat solution, varied from 1 to 8 ml., in duplicate series of 125-ml. low actinic, glass-stoppered separatory funnels. To one series add 2 ml. of a petroleum ether solution containing 15 micrograms of d,α-tocopherol per ml. Dilute the fat solution in all separatory funnels to 10 ml. with petroleum ether. Add 2 ml. of 0.2% ferric chloride reagent and 2 ml. of 0.5% 1,1′-bipyridine reagent. These reagents should be blown from pipets into the fat solution to ensure satisfactory mixing. Six minutes after addition of the ferric chloride, add 14 ml. of 86% ethyl alcohol (specific gravity 0.843 at 20°/20° C.) from a rapid delivery pipet. (A 15-ml. pipet with the tip cut off was found satisfactory for this purpose.) After 9 minutes run off the alcoholic layer into 40-mm. absorption cells and measure the absorbancy at exactly 10 minutes after the initial addition of the ferric chloride reagent, employing a Coleman Universal spectrophotometer set at a wave length of 515 mμ. Measure all absorbancies relative to the blank prepared by using 10 ml. of petroleum ether in place of the fat solution.

The tocopherol content of the aliquot employed for the determination can then be determined by the following formula:

$$\left(\frac{S}{ST - S}\right) \times 30 = \text{micrograms of tocopherol per aliquot}$$

where S is absorbancy due to tocopherol in the fat, and ST is absorbancy due to tocopherol in the fat solution + 30 micrograms of d,α-tocopherol.

The most accurate range was found to be from 20 to 120 micrograms of tocopherol per aliquot of fat solution.

Table III. Precision of Methods for Determination of Propyl Gallate, Butylated Hydroxyanisole, and Nordihydroguaiaretic Acid, and Tocopherol in Lard and Shortening

Substance	Mean of 6 Detns., %	Standard Deviation	99% Confidence Limits for Single Detn. (4.032 × Std. Deviation)
PG	0.01036	0.000047	±0.00019
PG[a]	0.00677	0.000046	±0.00019
BHA	0.02143	0.000247	±0.00100
BHA (after PG extraction)	0.01917	0.000028	±0.00011
NDGA	0.01029	0.000134	±0.00054
NDGA (with BHA)	0.01059	0.000063	±0.00025
BHA (with NDGA)	0.01942	0.000556	±0.00024
Tocopherol	0.1303	0.00291	±0.0117
Tocopherol (after BHA extraction)	0.1255	0.00337	±0.0135

[a] Addition of octanol to fat solution followed by extraction of PG with 1.67% ammonium acetate in 5% aqueous ethyl alcohol.

$$\text{Standard deviation} = \sqrt{\frac{\sum_{i=1}^{6} (x_i - \bar{x})^2}{5}}$$

where $\bar{x}$ = mean of 6 values, column 2
4.032 = value of student's t for $P = 0.01$ and degrees of freedom = (6–1) = 5

Table IV. Recoveries of Antioxidants Added to Fat Freed of Oxidizing Materials

Antioxidant		No. of Determinations	Amount Added, %	% Recovery
PG		4	0.01	98.0
NDGA		6	0.01	98.4
BHA		6	0.02	97.4
NDGA	combined in same sample	6	0.01	96.4
BHA	combined in same sample	6	0.02	97.0
BHA	after extraction of PG	6	0.02	96.9

REPRODUCIBILITY OF RESULTS

In order to determine the precision of the foregoing analytical procedures, six identical fat samples were analyzed for their respective antioxidant or antioxidants. In all cases the extract from a single sample was analyzed at three concentration levels, and the results were averaged and reported as a single figure. The results of six such determinations of each antioxidant, or group of antioxidants, were statistically analyzed and are shown in Table III. No attempt was made to employ the same amount of antioxidant in each test, although in all cases the concentration was in the range normally expected in foods.

It appears that adequate precision can be obtained by reporting a single value which is actually the average of three simultaneous determinations at different concentrations.

RECOVERY OF ANTIOXIDANTS

To obtain an accurate indication of the recovery of antioxidants in lard or shortening using the foregoing procedures, it was necessary to prepare a fat free of peroxides and other oxidizing materials. Oxidizing substances, if present in the fat, react with the antioxidant or antioxidants and thereby result in a low recovery.

Fat samples free of oxidizing materials were prepared by holding the fat containing approximately 0.05% of tocopherol at 80° C. for 10 minutes. If, on cooling, tocopherol was still present, it was concluded that there was an excess of tocopherol over oxidizing materials. In the case of fresh shortenings there is usually

sufficient natural tocopherol present and, therefore, none need be added. Table IV gives the recovery figures for various antioxidants added to fat previously freed of oxidizing materials. No recovery figure is given for tocopherol because this antioxidant is determined by means of an internal standard.

DISCUSSION

In the determination of propyl gallate with the ferrous tartrate reagent, the color reaction is very sensitive to variations in the pH. Optimum results were obtained when a pH of 7 was employed and in most cases the ammonium acetate buffer was sufficient to hold the pH close to 7. If, however, the solution is too acid, the color formation is incomplete and if the solution is alkaline the purple color has a tendency to fade. Gallic acid, tannic acid, and gallates other than propyl gallate may give a color reaction with ferrous tartrate at pH 7 which is identical to that produced with propyl gallate. However, tannic acid, gallic acid, and gallates other than propyl gallate are not usually employed as antioxidants in either Canada or the United States.

Gum guaiacum if present in lard or shortening will be extracted from a petroleum ether solution of a fat by 72% ethyl alcohol. Reducing substances in the gum react with ferric chloride plus 1,1′-bipyridine to produce a red color, which might be mistaken for butylated hydroxyanisole or nordihydroguaiaretic acid. Gum guaiacum can be readily detected by adding a few milliliters of the ferric chloride reagent to an aliquot of the 72% ethyl alcohol extract. The appearance of an intense blue color that rapidly fades indicates the presence of this material. This color should not be confused with the purple color produced by propyl gallate.

From the data in Table I, it can be seen that 2-*tert*-butyl-4-hydroxyanisole produces approximately 10% more color per unit weight than 3-*tert*-butyl-4-hydroxyanisole. However, in the case of commercial butylated hydroxyanisole preparations 1, 2, and 3, the variation is approximately 1%, measured at 30 minutes. Therefore, for investigational or control purposes the butylated hydroxyanisole preparation employed should be used as the standard.

The ferric chloride–1,1′-bipyridine reagent is not specific for butylated hydroxyanisole, nordihydroguaiaretic acid, or tocopherol and therefore could react with other reducing substances present in the fat. However, no such interference was encountered in any of the fat samples examined in this study.

The analytical method for the determination of tocopherols is not as precise as the methods for the other antioxidants. d,α'-Tocopherol was chosen as an arbitrary standard for this work. However, each of the four tocopherol isomers produces a different color intensity with ferric chloride and 1,1′-bipyridine (*9*). The procedure of Parker and McFarlane (*8*) was tried but was not sufficiently sensitive for the determination of the relatively small amounts of tocopherol present in lard and shortening. It was evident that large aliquots of fat would have to be employed in order to obtain sufficient tocopherol for a reasonably accurate analysis. The foregoing procedure was therefore devised, whereby a fat aliquot up to 320 mg. could be used compared to a maximum aliquot of 20 mg. in the Parker and McFarlane (*8*) procedure. The use of ferric chloride plus 1,1′-bipyridine in 100% ethyl alcohol ensured complete miscibility of these reagents with the petroleum ether solution of the fat, and thus permitted rapid reaction with the tocopherol. The subsequent addition of 14 ml. of 86% ethyl alcohol lowered the over-all alcohol content to 90%, which resulted in a separation of the alcoholic phase from the petroleum ether carrying with it the red ferrous bipyridine complex, while most of the fat remained in the petroleum ether phase. This procedure is intended only to give a reasonable estimate of the tocopherol present. Owing to the presence of traces of carotenoids in some shortenings, the results may be high unless the fat solution is treated to remove carotenoids before determining tocopherol (*8*).

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Butylated Hydroxyanisole in Lard and Shortening

Control Analysis

J. H. MAHON AND R. A. CHAPMAN

Food and Drug Laboratories, Department of National Health and Welfare, Ottawa, Canada

BUTYLATED hydroxyanisole (BHA) has been reported to be a very effective antioxidant for animal fats (*4*) and this information has stimulated interest in methods for its quantitative determination in lard and shortening. A modification of the Emmerie and Engel (*2*) ferric chloride plus 1,1′-bipyridine reagent has been employed satisfactorily (*7*) for the determination of this antioxidant, but this reagent is unstable to light and is not specific for butylated hydroxyanisole. Therefore, this study was initiated for the purpose of developing a reagent for the determination of butylated hydroxyanisole which does not exhibit these disadvantages.

Gibbs (*3*) found that 2,6-dichloroquinonechlorimide is a sensitive reagent for phenol and that the resulting blue indophenol is stable. Preliminary experiments indicated that this compound was highly specific for butylated hydroxyanisole among the antioxidants currently added to lard or shortening. Therefore, the reagent was studied more intensively with a view to adapting it for the quantitative determination of butylated hydroxyanisole.

EXPERIMENTAL

Previous work in this laboratory (*7*) established that 72% ethyl alcohol (by volume) was a satisfactory solvent for the ex-

The antioxidant butylated hydroxyanisole is extensively used in combination with propyl gallate or nordihydroguaiaretic acid for the stabilization of lard or shortening. Because butylated hydroxyanisole is usually present in the greater amount, a rapid and accurate analytical procedure would be of value in controlling the addition of antioxidant mixtures containing butylated hydroxyanisole to lard and shortening. A rapid colorimetric procedure has been developed for determination of butylated hydroxyanisole alone and in combination with propyl gallate or nordihydroguaiaretic acid. Butylated hydroxyanisole has been determined over the range of 0.01 to 0.02% with an average standard deviation for a single determination of 1.1% of the amount present. Recoveries are of the order of 97 to 99%. It is necessary to have the antioxidant preparation added to the lard or shortening for use as a standard.

traction of butylated hydroxyanisole from petroleum ether solutions of lard or shortening, and this concentration of ethyl alcohol was adopted as the solvent for all subsequent analyses.

Effect of pH. Gibbs (*3*) has indicated that the reaction between phenol and 2,6-dichloroquinonechlorimide is sensitive to pH. The effect of pH over the range of 6.8 to 10.0 on the rate of color formation between butylated hydroxyanisole and 2,6-dichloroquinonechlorimide was investigated, using a series of borate buffers (*1*). In every case 2 ml. of 0.01% 2,6-dichloroquinonechlorimide in absolute ethyl alcohol and 2 ml. of the appropriate aqueous buffer solution were added to the butylated hydroxyanisole in 12 ml. of 72% ethyl alcohol. After 15 minutes the absorbancy was measured at 610 mμ in a Beckman B spectrophotometer relative to the appropriate blank, with the results shown in Figure 1.

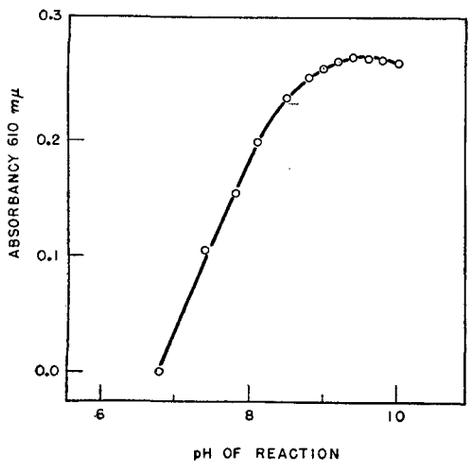

Figure 1. Effect of pH on 2,6-Dichloroquinonechlorimide–Butylated Hydroxyanisole Reaction

These data indicate that the maximum color formation between butylated hydroxyanisole and 2,6-dichloroquinonechlorimide occurs at pH 9.4. A dilute aqueous borax solution ($Na_2B_4O_7 10H_2O$) was used to buffer the reaction mixture at this pH in all subsequent analyses.

Effect of Time on Stability of Color. Experiments showed that maximum color formation and stability were obtained by using 12 ml. of 72% ethyl alcohol containing the butylated hydroxyanisole plus 2 ml. of 0.01% 2,6-dichloroquinonechlorimide in absolute ethyl alcohol and 2 ml. of 2% aqueous borax buffer solution. Under these conditions the resulting blue indophenol color reached maximum intensity in 15 minutes and was stable for over 5 hours. The absorbancy was measured against a blank with an Evelyn photoelectric colorimeter fitted with a 620 mμ filter. The effect of time on the absorbancy produced by butylated hydroxyanisole on reaction with the 2,6-dichloroquinonechlorimide–borax reagent employing the foregoing *conditions is* shown in Figure 2.

Specificity. A number of antioxidants were made to react with the 2,6-dichloroquinonechlorimide–borax reagent and after 15 minutes the wave length of maximum absorbancy was determined with a Beckman B spectrophotometer. The absorbancies were measured at 620 mμ with an Evelyn photoelectric colorimeter (Table I).

Table I. Wave Length of Maximum Absorbancy and Absorbanty Measured at 620 mμ for a Number of Antioxidants

Antioxidant	Wave Length of Max. Absorbancy, mμ	Absorbancy/γ at 620 mμ
3-BHA	610	0.0139
Commercial BHA		
No. 1[a]	610	0.0127
No. 2[a]	618	0.0092
2-BHA	655	0.0027
Hydroquinone	460	0.0020
Gum guaiacum	635	0.0014
NDGA	430	0.0003
Propyl gallate	435	0.0002
d,α-Tocopherol	No color produced	0.0000

[a] Mixtures of 2-BHA and 3-BHA.

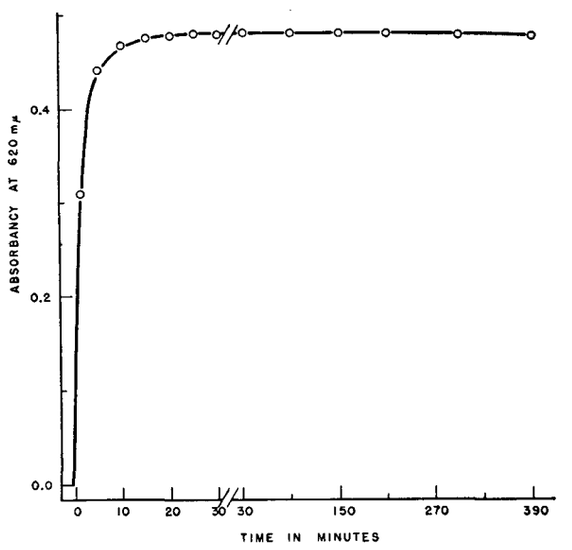

Figure 2. Effect of Time on Absorbancy Developed between Butylated Hydroxyanisole and 2,6-Dichloroquinonechlorimide–Borax Reagent

The data in Table I show that only gum guaiacum exhibits a maximum absorption at a wave length close to that given by butylated hydroxyanisole. Hydroquinone, nordihydroguaiaretic acid, and propyl gallate show maximum absorptions between 430 and 460 mμ. However, the latter antioxidants can interfere

Table II. Effect of Various Proportions of Propyl Gallate or Nordihydroguaiaretic Acid on Determination of Butylated Hydroxyanisole

Antioxidants Present	Absorbancy of Commercial BHA at 620 mμ, % No. 1	No. 2
BHA(10–50 microgram range)	100.0	100.0
BHA + 30% propyl gallate[a]	102.9	104.3
BHA + 50% propyl gallate	103.3	105.5
BHA + 25% NDGA	104.1	106.2
BHA + 50% NDGA	105.3	108.0

[a] Propyl gallate and NDGA expressed as % of BHA present.

in the determination of butylated hydroxyanisole, because they produce a slight absorption at 620 mμ. Commercial butylated hydroxyanisole No. 2 produces less absorbancy per microgram than does No. 1 because the former contains a smaller proportion of the 3-*tert*-butyl-4-hydroxyanisole isomer.

Effect of Propyl Gallate or Nordihydroguaiaretic Acid on Determination of Butylated Hydroxyanisole. The American Meat Institute Foundation has proposed an antioxidant mixture known as AMIF–72 (*5*), which contains 20% of butylated hydroxyanisole, 6% of propyl gallate, and 4% of citric acid in propylene glycol. This formula is widely used in commercial antioxidant preparations. From the data in Table I it appears that propyl gallate equal to 30% of the butylated hydroxyanisole present (as in the AMIF–72 type preparations) would produce only a relatively small error in the determination of butylated hydroxyanisole with the 2,6-dichloroquinonechlorimide–borax reagent. The effect of various proportions of propyl gallate or nordihydroguaiaretic acid on the determination of butylated hydroxyanisole as indicated by the absorbancy at 620 mμ is shown in Table II.

The data in Table II show that the addition of propyl gallate equal to 30% of the butylated hydroxyanisole results in an increase of 2.9 to 4.3% in the absorbancy measured at 620 mμ. The addition of propyl gallate equal to 50% of the butylated hydroxyanisole results in a further small increase. Similarly, the presence of nordihydroguaiaretic acid in mixtures with butylated hydroxyanisole produces a relatively small increase in absorbancy at 620 mμ as compared to butylated hydroxyanisole alone. As propyl gallate is frequently added to commercial butylated hydroxyanisole preparations, its presence will result in a characteristically small increase in the absorbancy measured at 620 mμ. However, if the same butylated hydroxyanisole preparation containing propyl gallate or nordihydroguaiaretic acid is used in the construction of the calibration curve, no error will result from this source.

REAGENTS

2,6-Dichloroquinonechlorimide, 0.01% solution in absolute ethyl alcohol. This reagent must be freshly prepared for optimum results.

Borax Buffer, 2.0% aqueous solution of $Na_2B_4O_7.10H_2O$.

Petroleum Ether, Skellysolve H.

Alcohol, 72% ethyl alcohol by volume.

PROCEDURE

Place 10 grams of the fat in a 500-ml. separatory flask and dissolve in 50 ml. of petroleum ether (Skellysolve H). Extract the petroleum ether solution of the fat by shaking the contents of the flask with three separate 25-ml. aliquots of 72% ethyl alcohol for 3 minutes per extraction. Make a fourth extraction, using 60 ml. of 72% ethyl alcohol and shaking for 1 minute. Combine the four extracts, dilute to a suitable volume (150 to 300 ml.) with 72% ethyl alcohol, and filter through two Whatman No. 54 filter papers. The clear alcoholic extract contains the butylated hydroxyanisole plus propyl gallate or nordihydroguaiaretic acid. Place three suitable aliquots (1 to 12 ml.) of the extract in separate 18-mm. colorimeter tubes and dilute to 12 ml. with 72% ethyl alcohol. Add to each tube 2 ml. of freshly prepared 0.01% 2,6-dichloroquinonechlorimide reagent. Mix the contents of the tubes, add 2 ml. of 2% aqueous borax solution, and mix the contents again. Prepare a blank containing 12 ml. of 72% ethyl alcohol and the reagents. After 15 minutes measure the absorbancy relative to the blank in an Evelyn photoelectric colorimeter fitted with a 620 mμ filter. Calculate the amount of butylated hydroxyanisole by reference to a calibration curve prepared over the range of 10 to 50 micrograms of butylated hydroxyanisole per tube, using the same butylated hydroxyanisole preparation that was added to the fat. It is necessary to prepare a new calibration curve for each type or batch of antioxidant preparation used.

REPRODUCIBILITY

Three samples of shortening were prepared containing 0.0200, 0.0150, and 0.0100% of butylated hydroxyanisole, respectively. In all cases the butylated hydroxyanisole was added as a commercial antioxidant preparation containing 20% butylated hydroxyanisole, 6% propyl gallate, and 4% citric acid in propylene glycol. Each of the shortening samples was analyzed four times for butylated hydroxyanisole, employing the foregoing procedure. The butylated hydroxyanisole content of the samples was calculated by means of calibration curves prepared using the same antioxidant preparation added to the shortening. The recovery of butylated hydroxyanisole and the reproducibility of the analytical data are shown in Table III.

The data in Table III indicate that recoveries of butylated hydroxyanisole of the order of 97 to 99% can be obtained. The apparent loss of 1 to 3% of the butylated hydroxyanisole added might be attributed in part to the destruction of some of the butylated hydroxyanisole by the small amount of fat peroxides present. The estimated standard deviation for a single determination is of the order of ±1% of the butylated hydroxyanisole present in the sample.

Table III. Recovery of Butylated Hydroxyanisole Added to Shortening and Reproducibility of Results

BHA Added, %	Av. BHA Found, %	Estimated Standard Deviation for Single Estimation
0.0200	0.0198	0.0002
0.0150	0.0146	0.0002
0.0100	0.0097	0.0001

DISCUSSION

Lundberg (*6*) employed a 2,6-dichloroquinonechlorimide reagent in the presence of a borax buffer for the determination of nordihydroguaiaretic acid. Lundberg's reaction mixture is predominantly aqueous and the borax buffer is added to the nordihydroguaiaretic acid solution before the 2,6-dichloroquinonechlorimide reagent. This procedure results in the formation of a red color with a maximum absorption at 545 mμ. However, if the order of adding Lundberg's reagents is reversed, no red color is formed. Using the procedure recommended in this paper, no red color is formed upon the addition of the 2,6-dichloroquinonechlorimide reagent to an alcoholic solution of nordihydroguaiaretic acid followed by the addition of the borax buffer. Apparently, the order of addition of the reagents is responsible for the different results obtained with these two procedures.

The absorbancy produced with the 2,6-dichloroquinonechlorimide–borax reagent is 5.2 times as great with 3-*tert*-butyl-4-hydroxyanisole as with an equal weight of 2-*tert*-butyl-4-hydroxyanisole. As the proportion of 3-*tert*-butyl-4-hydroxyanisole to 2-*tert*-butyl-4-hydroxyanisole is variable in commercial butylated hydroxyanisole preparations, the 2,6-dichloroquinonechlorimide–borax reagent cannot replace the ferric chloride plus 1,1′-bipyridine reagent (*7*) for the analysis of unknown butylated hydroxyanisole preparations. In order to employ the 2,6-dichloroquinonechlorimide–borax reagent in the determination of butylated hydroxyanisole, it is essential to use the same antioxidant preparation that is present in the lard or shortening to obtain the calibration curve.

The 2,6-dichloroquinonechlorimide–borax reagent has been found to be superior to the ferric chloride plus 1,1′-bipyridine reagent for the determination of butylated hydroxyanisole:

It is unaffected by strong light.

Maximum color intensity is obtained in 15 minutes.

The color is stable for over 5 hours.

This reagent is relatively specific for butylated hydroxyanisole among the antioxidants currently added to lard or shortening.

Extraneous reducing agents are unlikely to produce colored complexes with this reagent. Consequently, rigid purification of the petroleum ether and ethyl alcohol used as solvents for the extraction and analysis of butylated hydroxyanisole is not necessary, as it is when the ferric chloride plus 1,1′-bipyridine reagent is used.

This reagent permits the determination of butylated hydroxyanisole in the presence of propyl gallate or nordihydroguaiaretic acid, provided the original antioxidant preparation can be used to construct a calibration curve.

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Determination of Titanium in Rocks and Minerals

Mercury Cathode Polarographic Method

R. P. GRAHAM AND J. A. MAXWELL[1]

Hamilton College, McMaster University, Hamilton, Ontario, Canada

The procedures described were developed to provide a method for the determination of titanium in rocks and minerals that is free of some of the disadvantages of the conventional colorimetric method. After electrolysis of a solution of the sample in a mercury cathode cell to remove iron and other interfering ions, titanium is determined polarographically. The supporting electrolyte, 1.0 *M* in tartaric acid and 0.5 *M* in sulfuric acid, permits the recording of a well-formed polarographic wave, the height of which is directly proportional to the concentration of titanium in the solution. Of the foreign elements not removed in the mercury cathode cell, only uranium interferes with the subsequent polarographic procedure. The method is of good precision and yields values in close agreement with those given by the colorimetric method using hydrogen peroxide. Although not as sensitive as the colorimetric method, it is less subject to errors arising from foreign ions and is in certain respects more convenient.

TITANIUM is the ninth most abundant element of the earth's crust, in which it occurs to the extent of approximately 0.5%. Its determination in any rock or mineral is therefore a necessity if the analysis is to be considered complete.

Titanium is usually determined in rocks and minerals by making use of the yellow color that is developed by the addition of hydrogen peroxide to a solution of a titanium(IV) salt (*15*). This method, however, is subject to interferences (*14*) from elements that form colored solutions (such as iron, chromium, and nickel), elements that form colored compounds with hydrogen peroxide (such as vanadium, molybdenum, and cerium), and ions that bleach the color of the complex (such as fluoride and large concentrations of alkali salts or phosphate). All these interferences can be overcome by simulating conditions in the standard, but this is a very troublesome procedure that involves detailed knowledge of the nature of the material being analyzed.

Zeltzer (*17*) suggested a polarographic method for the determination of titanium in titaniferous minerals by fusing the powdered sample with potassium hydrogen sulfate, dissolving the melt in dilute sulfuric acid, and then polarographing the resultant solution. He reported good waves in this medium, but this could not be substantiated by Adams (*1*) nor Potts (*12*).

In a summary (1941) of polarographic data on titanium, Kolthoff and Lingane (*4*) stated that acid solutions of tartaric or citric acid are preferable as a supporting electrolyte. In such solutions the cathodic diffusion current is directly proportional to the concentration of titanium(IV) ions.

Adams (*1*), in his work on the determination of titanium in clays and clay products, used as supporting electrolyte a 0.5 *M* solution of sulfuric acid saturated with sodium oxalate and containing 8% urea as a maximum suppressor. He reported that the concentrations of both titanium(IV) and iron(III) affect the position of the half-wave potential of the titanium, but Lingane and Vandenbosch (*8*) disagree and show that the half-wave potential remains constant. They also state that the urea does not function satisfactorily as a maximum suppressor when the titanium concentration exceeds a certain value.

Potts (*12*) found that satisfactory waves could be obtained in a sulfuric-tartaric acid supporting electrolyte, without a maximum suppressor, and he determined titanium in paint pigments using this medium.

The present paper describes a polarographic method for the determination of titanium in rocks and minerals that is less subject to interference than the colorimetric method. A mercury cathode cell is used to remove elements that would cause interference in the polarographic procedure.

APPARATUS AND REAGENTS

Apparatus. A Heyrovský polarograph, Model XI (E. H. Sargent and Co.), was used throughout this work. The capillary was of marine barometer tubing supplied by E. H. Sargent and Co., and was cleaned, when necessary, by immersing it in hot nitric acid. The m and t values of the capillary in the supporting electrolyte were determined at 25.0° ± 0.2° C. by the method of Lingane and Kolthoff (*6*). At an applied potential of −0.30 volt *vs.* the saturated calomel electrode, the drop time, t, was 5.19 seconds, the rate of flow of mercury, m, was 1.34 mg. per second, and $m^{2/3}t^{1/6}$ was 1.59 mg.$^{2/3}$sec.$^{-1/2}$ [The variation of $m^{2/3}t^{1/6}$ with applied potential, over the range 0.0 to −1.1 volts, was found to be in very close agreement with the relative values given

[1] Present address, Department of Geology and Mineralogy, University of Minnesota, Minneapolis, Minn

by Kolthoff and Orlemann (*5*).] The cell used was of the H type, substantially that described in the literature (*7*), one arm of which contained the saturated calomel reference electrode (*11*).

The mercury cathode cell used was identical to that designed by Ford (*10*); this cell is of the type described by Rabbitts (*13*).

Except where indicated in Table II, all colorimetric measurements were made using a Coleman Universal spectrophotometer, Model 11, at 420 mμ, as recommended by Sandell (*14*).

Reagents. All reagents were tested for the presence of titanium, both colorimetrically and polarographically. No positive tests for titanium were encountered.

The mercury used in the mercury cathode cell was purified by bubbling for 10 hours with 10% nitric acid and then for 10 hours with distilled water. Mercury for the dropping electrode was further purified by two distillations.

REMOVAL OF INTERFERING IONS

The composition of most rocks and minerals is complex and the presence of interfering elements is to be expected. Paramount among possible interferences in the polarographic determination of titanium is iron(III) which, in 1.0 *M* solutions of most acids, begins to discharge at zero applied potential and thus will interfere with the titanium wave (*3*). [Since the present paper was written, the authors learned that Vandenbosch (*16*) has found that a supporting electrolyte of sulfuric acid (10%) and potassium oxalate (20 grams per liter) is suitable for the polarographic determination of titanium in the presence of iron.] It has been shown (*10*) that iron, copper, and chromium can be removed conveniently by electrolysis of the solution in a mercury cathode cell, with quantitative retention of the titanium in the electrolyte. This method has much to recommend it in simplicity and rapidity.

CHOICE OF SUPPORTING ELECTROLYTE

The sulfuric acid–sodium oxalate–urea method of Adams (*1*) was investigated but, although well-formed waves were obtained, the appearance of maxima and the unfavorable comments of Lingane and Vandenbosch (*8*) militated against its use. Good waves (Figure 1) were obtained with a tartaric-sulfuric acid supporting electrolyte, and this was chosen as the medium. No maximum suppressor was found necessary.

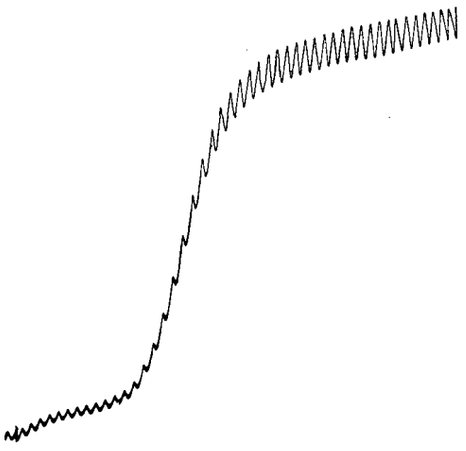

Figure 1. Typical Polarographic Wave for Titanium, Obtained with Sample of Ilmenite

For a given concentration of titanium(IV) ions, an increase in the concentration of the tartaric acid in the supporting electrolyte was found to cause a pronounced increase in the height of the polarographic wave, as well as a slight change in its shape. Investigation showed that a 1.0 *M* solution of tartaric acid in 0.5 *M* sulfuric acid was the most satisfactory supporting electrolyte. Potts (*12*) used approximately 0.8 *M* tartaric acid, in sulfuric acid solution, in his work with paint pigments.

Table I. Effect of Foreign Ions

Soln. No.	Elements Added *Mg./Ml.*	Ti Taken *Mg./Ml.*	Ti Found *Mg./Ml.*
1	Al (0.06)	0.060	0.061
2	Al (0.20)	0.060	0.061
3	Al (3.0)	0.060	0.060
4	Al (1.2) Mg (2.0) Na (2.0) K (2.0) Ca (0.8)	0.050	0.048
5	Al (2.6) Mg (1.0) Na (1.0) K (1.0) Ca (0.4)	0.050	0.049
6[a]	La (0.02) Ce (1.4)	0.050	0.049
7	Li (0.2)	0.050	0.050
8	Zr (0.1)	0.050	0.050
9	Th (0.2)	0.050	0.050
10	U (0.02)	0.050	0.067
11	Nb (0.2)	0.050	0.050
12	Ta (0.2)	0.050	0.049
13	Nb (0.02) Ta (0.02)	0.050	0.050
14	Nb (0.05) Ta (0.05)	0.050	0.049
15	Nb (0.5) Ta (0.5)	0.050	0.050

[a] This solution undoubtedly contained other lanthanide elements.

SIZE AND DECOMPOSITION OF SAMPLES

The amount of the sample (ground to at least −100 mesh) taken for analysis is governed by the concentration of titanium present. Under the conditions used in the analysis, the most convenient range of concentration, in the final solution, was found to be 1.6×10^{-3} to 1.0×10^{-4} *M* with respect to titanium. On the basis of a 1-gram sample in a final volume of 100 ml., this corresponds to 0.77 to 0.05% of titanium. Lower concentrations of titanium can be determined by working at increased instrumental sensitivity or by taking a larger sample.

The sulfuric-nitric-hydrofluoric acid method outlined by Lundell and Hoffman (*9*), for the decomposition of bauxite samples in which silica is not to be determined, was used, except that nitric acid was omitted from the mixed acids. This method was chosen because it was desired to eliminate silica (which made up a large proportion of some of the samples) and to avoid, as much as possible, the introduction of foreign salts.

METHOD OF ANALYSIS

The sulfuric acid solution of the sample was diluted to approximately 75 ml., and transferred to a mercury cathode cell in which it was electrolyzed at 8 to 10 amperes (0.15 ampere per sq. cm.) and 4 to 6 volts. The electrolysis was continued until only a trace quantity of iron could be detected in the electrolyte by spot-test procedures using potassium thiocyanate and 2,2′-bipyridine. At this stage, as a precautionary measure, the walls of the cell were washed down with distilled water and the electrolysis was continued for another 5 to 10 minutes. Although the time of electrolysis varied with the concentration of iron originally in solution, usually 20 to 30 minutes were sufficient to reduce the concentration of iron to a value such that no interference was caused in the subsequent polarographic procedure.

The electrolyzed solution was quantitatively removed from the cell and 15 *M* aqueous ammonia was added until the solution was just basic to litmus. Sufficient 18 *M* sulfuric acid and tartaric acid, the latter as a solution in 0.5 *M* sulfuric acid, were added so that on necessary dilution and adjustment to volume, the final solution was 0.5 ± 0.1 *M* with respect to sulfuric acid and 1.00 ± 0.05 *M* with respect to tartaric acid. A portion of this solution was then decanted into the polarographic cell. Some turbidity in the solution at this stage causes no difficulty in the polarographic procedure, but in the present investigation the solutions were filtered because, for purposes of comparison, a colorimetric determination of the titanium was also to be made.

Nitrogen which had been purified (by passing it through sodium pyrogallate and then distilled water) was used to remove

oxygen from the solutions prior to polarographing them. The temperature of the cell solutions was maintained at 25.0° ± 0.5° C. by means of a thermostatically controlled water bath.

The solutions were polarographed from 0.0 to −0.8 volt and the half-wave potential was found to be approximately −0.3 volt *vs.* S.C.E. Each solution was polarographed four times and each of the four polarograms, which were photographically recorded, was measured once by the slope-intercept method.

Titanium concentrations were assessed by reference to a straight-line calibration curve relating the concentration of titanium to wave height. The calibration was carried out using solutions prepared from Bureau of Standards titanium dioxide No. 154.

For the samples studied, the half-wave potentials ranged from −0.22 to −0.44 volt with the majority occurring at −0.30 ± 0.02 volt (*vs.* S.C.E.). Although in all these determinations the concentration of sulfuric acid was constant (0.5 *M*), in some separate experiments it was observed that, with a constant concentration of titanium(IV) and 1.0 *M* tartaric acid, the value for the half-wave potential increased with decreasing concentration of the sulfuric acid in the supporting electrolyte, ranging from −0.26 volt for 0.75 *M* sulfuric acid to −0.33 volt for 0.25 *M*. The value given by Kolthoff and Lingane for the half-wave potential of titanium(IV), in saturated tartaric acid solution, is −0.44 volt *vs.* S.C.E. (*4*).

EFFECT OF FOREIGN IONS

Electrolysis in the mercury cathode cell removes iron, copper, chromium, and numerous other elements (*10*). After such an electrolysis the solution of a rock or mineral may still contain, in addition to titanium(IV) ions, significant concentrations of the elements listed in Table I. In order to study the possible interference of these ions in the polarographic determination of titanium, standard titanium solutions were prepared that contained one or more of the possible interfering ions in concentrations at least as high as might be expected in the analysis of a rock or mineral. These solutions were then polarographed by the recommended procedure. The results are compared, in Table I, with the known titanium concentration of the solution. In addition, composite solutions containing similar concentrations of all the elements listed in Table I (with the exception of uranium) were polarographed with similar results.

Zeltzer (*17*) found aluminum to be without effect on the titanium wave height, but Potts (*12*) and Adams (*1*) report that a high aluminum content depresses the diffusion current of the titanium. From Table I it is evident that in the authors' experiments the effect of the aluminum is not significant.

Of the elements investigated, only uranium introduces a significant error. Study showed that a wave due to uranium occurred at a half-wave potential of approximately −0.17 volt *vs.* S.C.E., a value in close agreement with that (−0.18 volt) given by Harris and Kolthoff (*2*) for the reduction of uranium(VI) to uranium(V).

Particular attention was paid to possible interference from niobium and tantalum because of their close geochemical relationship with titanium, but, as is evident from Table I, they do not interfere in the polarographic method.

PRECISION OF MEASUREMENTS

In order to assess the uncertainty in the measurement of wave heights, experiments were carried out which involved repeated measurements of the height of a single wave, repeated measurements of the heights of different waves obtained with the same solution, and repeated measurements of the heights of waves obtained with different aliquot samples of the same solution.

Four aliquot samples were taken from a standard titanium solution and diluted so that the final titanium concentration of each was 0.050 mg. per ml. Each solution was then polarographed four times under the recommended conditions and then each of the sixteen waves was measured ten times by the slope-intercept method.

The standard deviations for the 16 sets of 10 measurements ranged from 0.2 to 0.6 mm., with an average standard deviation of 0.3 mm. These values give a measure of the personal error in measuring the height of a wave of 59 mm. and do not include uncertainties due to the volumetric or polarographic procedures.

When the results are considered in groups of 40, the standard deviations reflect both the personal or measuring uncertainty and the polarographic or instrumental uncertainty. In the four sets of this kind of measurement, the standard deviations ranged from 0.7 to 1.2 mm. with an average standard deviation of 1.0 mm.

When the entire 160 measurements were grouped together, the standard deviation rose slightly (to 1.1 mm.), reflecting a small uncertainty due to the making up to volume of different aliquot samples (the volumetric uncertainty). The uncertainty of a measured value occasioned by this factor is thus only one seventh of that due to the polarograph, and one third that due to personal factors in measuring.

Of the 160 measurements, the average value (59.1 mm.) was equivalent to 0.050 mg. of titanium per ml. (10^{-3} *M*), the lowest value (57.5 mm.) to 0.049 mg. per ml., and the highest value (61.5 mm.) to 0.052 mg. per ml. Thus the maximum deviation of a measured value from the average of 160 values was 4%—a normal reproducibility for the polarographic method.

ANALYSIS OF ROCKS AND MINERALS

The results of polarographic determinations of titanium on a variety of rocks and minerals are shown in Table II, in comparison with the values obtained by the colorimetric method.

The agreement between the two methods and the agreement between duplicate samples using the same method are good, in view of the difficulty of securing representative samples of such heterogeneous substances (see the spread in Bureau of Standards values in Table III).

In Table III a comparison is given of the results obtained by the

Table II. Comparison of Polarographic and Colorimetric Methods

Samples	% Titanium Dioxide	
	Polarographic	Colorimetric
Limestone	0.13	0.13
	0.13	0.12
Glass sand	0.08	0.10
	0.08	0.09
Magnetite	0.12	0.12
	0.13	0.12
Euxenite	0.40	0.42
	0.38	0.39
Syenite	0.22	0.20
	0.21	0.18
Granodiorite[a]	0.21	0.23
Granite[a]	0.22	0.22
Alkali syenite[a]	0.21	0.22
Plastic clay	1.32	1.38
	1.29	1.28
Flint clay	2.39	2.29
	2.32	2.30
Ilmenite	37.3	36.8
	38.5	35.9
Sphene	30.0	30.8
	31.8	29.1

[a] Colorimetric values for these samples were obtained with a Fisher AC Model electrophotometer, using a 425 B filter, rather than the Coleman instrument. Calibration curves for both instruments were constructed using solutions prepared from Bureau of Standards titanium dioxide No. 154.

Table III. Comparison of Polarographic Values with Those of Bureau of Standards

Sample	Titanium Dioxide, %		
	Bureau of Standards		Polarographic, average[a]
	Spread	Average	
Limestone 1a	0.11–0.20	0.16	0.13 ± 0.00
Glass sand 81	0.089–0.112	0.095	0.077 ± 0.02
Magnetite 29a	not stated	0.15	0.13 ± 0.01
Plastic clay 98	1.35–1.50	1.43	1.31 ± 0.02
Flint clay 97	2.28–2.44	2.38	2.36 ± 0.04

[a] Average of two separate determinations with maximum deviation.

proposed polarographic method and the values given on the certificate supplied with the samples by the Bureau of Standards. For the type of substance being analyzed, the correspondence is satisfactory.

DISCUSSION

The proposed mercury cathode–polarographic method for titanium has been proved valid with a variety of rocks and minerals. A study has been made of elements that are not removed by the mercury cathode separation and yet are likely to be encountered in significant concentrations in rocks and minerals; of these, only uranium raises a problem. The mercury cathode method of removing interfering ions, in addition to avoiding the introduction of foreign salts, does not require much attention during operation, thus freeing the analyst for other work. The proposed method has certain advantages of simplicity and rapidity over the conventional colorimetric method, in that it does not require the preparation of a standard similar in composition to the sample being analyzed, nor filtrations to remove turbidity. In contradistinction to the colorimetric method, no significant error is caused by the presence of a high concentration of alkali salts or cerium, or of traces of fluoride ion.

The method does not equal the colorimetric method in sensitivity, and thus it seems unlikely to serve for the determination of trace quantities of titanium, unless large samples are used. It can, nevertheless, be of advantage in routine determinations, where its reduced sensitivity is compensated by its increased rapidity and convenience. It is hoped to adapt the method to the determination of titanium in steels and other alloys.

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Saponification of Difficultly Saponifiable Esters

W. E. SHAEFER AND W. J. BALLING, *Hercules Experiment Station, Hercules Powder Co., Wilmington, Del.*

The increased use of complex esters of rosin has made it necessary to develop a simple and accurate drastic saponification method suitable for routine use. Previously published methods which gave acceptable accuracy and precision required rather elaborate apparatus. Improved conditions eliminate or minimize the difficulties formerly encountered. Atmospheric oxygen is excluded by adding a small amount of phenetole to the diethylene glycol solution of potassium hydroxide used as the reagent. Alkali-resistant glass reaction flasks are recommended because they are attacked by the reagent only to a negligible extent. Although either a double indicator method or the usual difference method may be used to obtain results which are concordant within two units, the former procedure may be expected to give results of greater precision and accuracy. The methods described are applicable not only to resins but also to difficultly saponifiable esters in general.

WITH the increased use of complex esters of rosin, the need for a simple drastic method for their quantitative saponification has been felt keenly. Furthermore, a standard method that is applicable to such resins will also be of considerable value in the examination of certain difficultly saponifiable plasticizers.

The rapid diethylene glycol saponification method of Redemann and Lucas (*2*), in which saponification is completed in 2 minutes or less, yielded excellent results when applied to the analysis of easily saponifiable esters. For difficultly saponifiable esters, Shaefer and Piccard (*4*) used either a saponification reagent formed by dissolving sodium in *tert*-butyl alcohol or one which consisted of sodium methoxide dissolved in methanol and cyclohexanol. The first of these reagents gave theoretical saponification values for dibornyl phthalate, and the second, although not applicable to ester gum, the glycerol ester of rosin, yielded excellent results when applied to other difficultly saponifiable esters of abietic acid. Unfortunately, the latter method requires both the use of a high-temperature bath and the passage of nitrogen through the apparatus and thus is unsuited for rapid routine use.

By increasing the reaction time, Hall, Holcomb, and Griffin (*1*) successfully applied the diethylene glycol method of Redemann and Lucas to the moderately difficultly saponifiable isomeric menthol acetates. This procedure was used routinely in Hercules laboratories during a number of years for the saponification of certain esters. However, its use for the quantitative saponification of the more difficultly saponifiable materials such as rosin esters was never satisfactory, primarily because the necessary reaction time of 1 to 2 hours caused the glass to be seriously attacked by the reagent and an appreciable amount of alkali was thus consumed. Other errors were introduced at times by using a reagent having too low a boiling temperature, one having such a high viscosity that its accurate delivery from a pipet is impossible,

or one that is partially decomposed on being subjected to prolonged refluxing. The precision and accuracy of the results were poor and determinations had to be repeated frequently.

In the procedure more recently developed, the foregoing difficulties are either eliminated or minimized. Alkali-resistant flasks (substantially boron-free) are used, even though they are more expensive and fragile; titrations are made by the double-indicator method of Rieman (*3*), though the usual difference method may also be used. The reagent consists of an approximately 0.8 *N* solution of potassium hydroxide in about 92% diethylene glycol and about 8% phenetole by volume.

As the first step in forming this reagent, a solution of potassium hydroxide in diethylene glycol is prepared by mechanically shaking or rotating at room temperature a bottle containing a mixture of these compounds. By following this procedure, instead of effecting solution by heating at a temperature below 130° C. as recommended by Redemann and Lucas (*2*), a completely colorless solution is obtained. The phenetole is added to this solution both to increase the solvent power of the reagent and to provide a blanket of vapor above the reaction mixture and thus exclude atmospheric oxygen from it. If protected from sunlight, this reagent remains substantially colorless for several weeks.

DOUBLE-INDICATOR METHOD

Apparatus. Erlenmeyer flasks, 300-ml., alkali-resistant glass, $\overline{\$}$ 24/40 joint, Corning Glass Works, Catalog No. 75000. Allihn condensers, borosilicate glass, $\overline{\$}$ 24/40 joint.

Reagent. Diethylene glycol–potassium hydroxide–phenetole solution, 0.8 *N*, containing about 2 ml. of phenetole in 25 ml. of reagent. Add 48 grams of potassium hydroxide (conforming to ACS specifications) to a bottle containing 800 ml. of substantially anhydrous diethylene glycol. Insert a ground-glass stopper which has been lubricated with diethylene glycol. Shake the bottle vigorously at room temperature until solution is effected, usually for 2.5 to 3 hours. Add 70 ml. of phenetole and shake the mixture manually for a moment to form a homogeneous colorless solution. Store this reagent in an amber-colored bottle.

Procedure. Place an approximately 2-gram sample, weighed to the nearest 0.001 gram, in a 300-ml. alkali-resistant flask and add from a Lowy pipet 25 ml. of reagent, which need not be measured accurately. Also add a few particles of 10-mesh silicon carbide. Lubricate the ground-glass joint of the condenser with a few drops of diethylene glycol.

Attach the flask to a reflux condenser, place it on a hot plate, and heat for 2 hours at such a rate that the solution will reflux gently. The reaction temperature will then be about 175° C. Take special care to see that the flask is not heated to a higher temperature than necessary for gentle refluxing, either at the beginning of the determination or later. Overheating results in the darkening of the reaction mixture due to the reaction of diethylene glycol with alkali; the products of this reaction cause blank values to be high and analytical results to be low and discordant.

At the end of the heating period, add sufficient ethyl alcohol to the top of the condenser so that a few drops will run down into the flask, thus filling it with alcohol vapor. Raise the hot flask onto a suitable Transite support, cautiously add 25 ml. of previously neutralized ethyl alcohol, and remove the flask from the condenser at once. Under these conditions the flask and condenser can be separated easily in spite of their different coefficients of expansion. Insert a stopper and cool the flask in cold water for a few minutes.

In this method the saponification value is calculated from the quantity of potassium salts formed in the saponification reaction. The accurate neutralization of the reaction mixture and the titration of the potassium salts are accomplished as follows:

Add thymol blue indicator, titrate the solution slightly past the end point with *N* alcoholic hydrochloric acid, and then back exactly to the end point with 0.25 *N* alcoholic sodium hydroxide without measuring the amounts of acid and alkali used. Add 10 drops of a 0.5% alcoholic solution of tetrabromophenol blue. Using 0.25 *N* alcoholic hydrochloric acid, titrate the solution of potassium salts to the greenish-yellow color chosen as the end point. In case the reaction mixture is very dark colored, adjust the pH value of the solution at 11.0 and then titrate the potassium salts electrometrically to a pH of 3.5. These pH values are chosen because in the authors' experience they are the correct ones to use in titrating an alcoholic solution.

Determine blank values on the reagent by the foregoing procedure. This is required by the presence in the reagent of carbonate and of impurities formed by the reaction of diethylene glycol with potassium hydroxide. Blank values are usually less than 1 ml. of 0.25 *N* alcoholic hydrochloric acid.

From the corrected volume of 0.25 *N* alcoholic hydrochloric acid solution used in the final titration, calculate the saponification number in the usual manner.

If desired, the foregoing procedure can be modified by performing the titration in an alcohol-water solution in which the ratio of alcohol and water is substantially constant. If this is done, the soap should preferably be titrated with aqueous instead of alcoholic hydrochloric acid. The correct pH values for the electrometric titration end points would have to be determined experimentally. In accordance with the method of Rieman, 10 ml. of benzene can be added to dissolve any precipitate of an organic acid which may be formed because of the addition of water.

Analytical Results. The saponification numbers found for two rosin esters under different conditions of time and sample weight are contained in Table I. On the basis of these results it is concluded that a 2-hour reaction time gives higher and presumably more nearly correct results than a 1-hour reaction time, but that heating the reaction mixture for a still longer time is not helpful. As is to be expected, the use of 2-gram samples gives somewhat more concordant results.

When saponified for 2 hours by the foregoing procedure, purified methyl dehydroabietate was found to have saponification numbers of 177.6 and 177.2. When the reaction time was 4 hours, the results were 178.2 and 177.2. The theoretical value is 178.4. Other abietic esters of high purity were not available. By this method the saponification number of N wood rosin was found to be 176, whereas the usual procedure with alcoholic potassium hydroxide reagent yields results of about 167.

Discussion. The foregoing drastic saponification method possesses the following advantages: Blank values are small and need to be determined only occasionally; the rather viscous reagent need not be measured accurately; and results are more accurate and concordant because only the potassium salt formed in the reaction is titrated.

In the course of making 105 determinations the total time of refluxing was 227 hours. The flasks used in this work decreased in weight by 0.202 gram, which represents an average loss of 0.0009 gram per flask per hour of heating. This cannot affect the results to a significant extent.

In a number of determinations a slow current of nitrogen was passed into the flasks before they were heated, throughout the heating period, and while they were being cooled. This precaution was without effect and demonstrated that no oxidation was occurring under the recommended conditions.

USUAL DIFFERENCE METHOD

In order to have a slightly more rapid procedure available for the routine determination of saponification numbers, determinations were made by the usual difference method. The same reagent and alkali-resistant flasks were used and the same saponification procedure was followed. However, the reagent

Table I. Saponification of Rosin Esters by Double-Indicator Method

Time of Refluxing, Hours	Approximate Size of Sample, Grams	Saponification Numbers: Ester gum[a]	Saponification Numbers: Staybelite ester 10[b]
1	1	167.2	165.7
1	1	167.9	164.5
1	2	168.9	166.3
1	2	166.8	165.3
2	1	171.2	165.7
2	1	170.9	166.3
2	2	170.2	166.2
2	2	170.1	165.5
3	2	170.8	164.9
3	2	170.8	166.3

[a] Glycerol ester of rosin.
[b] Glycerol ester of hydrogenated rosin.

Table II. Saponification of Abalyn[a] by Usual Difference Method

Saponification Numbers	
1-hour refluxing	2-hour refluxing
165.8	165.4
168.4	165.3
165.2	165.8
164.6	164.5
167.5	164.6
165.6	166.5
Av. 166.2	165.4

[a] Methyl ester of rosin.

was measured from an automatic dispensing pipet (Ace Glass, Inc., Catalog No. 8001) having a tip 3 mm. in diameter and the blank solutions and reaction mixtures were titrated by using phenolphthalein as the indicator. The results shown in Table II indicate that Abalyn, the methyl ester of rosin, is completely saponified in 1 hour under the selected conditions. However, the results found after 2 hours are slightly more concordant than those found after 1 hour. When Abalyn is heated with the usual alcoholic potassium hydroxide reagent for an hour, the saponification values found are in the range of 20 to 25—i.e., about 15% of the correct value.

A second compound, terpinyl acetate, was analyzed by both the drastic and the usual alcoholic potassium hydroxide procedures. The results in Table III show that the latter procedure gives low results, while saponification by the drastic method is essentially complete at the end of one hour.

Table III. Saponification Number of Terpinyl Acetate[a]

(Comparison of methods)

Drastic Method		Alcoholic KOH,
1 hour	2 hours	1 Hour
256.3	257.6	220.1
225.6	257.2	222.3

[a] Technical grade, Eastman Kodak Co. Theoretical saponification number of terpinyl acetate is 286.

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Color Reactions of Thiophene Derivatives

And of Other Compounds with a Ceric Nitrate Reagent

H. D. HARTOUGH[1]

Research and Development Department, Socony-Vacuum Laboratories, Paulsboro, N. J.

Work undertaken to determine whether other types of sulfur compounds and closely related heterocyclic compounds would give color tests similar to those produced by thiophene with hexanitratoammonium cerate reagent showed that pyrroles, indole, carbazole, and phenol gave color tests similar to those given by thiophene derivatives. A new color test for organic sulfides appears to be general for all types of sulfides, and color reactions for 21 thiophene derivatives have been determined. Other types of sulfur compounds do not give interfering color tests. Color reactions of the nine homologous methylthiophenes indicate that each compound exhibits a specific color sequence. Thianaphthene and dibenzothiophene give the same color reaction as thiophene, and an attempt has been made to explain color reactions of thiophene compounds on basis of formation of a molecular complex.

A PREVIOUS paper (*3*) indicated that positive color reactions with a hexanitratoammonium cerate reagent were produced when the thiophene nucleus possessed an α-hydrogen, α-methyl, or α-acetyl group. The data presented in Table I indicate that these conclusions were premature and the principles are much more involved than previously suspected. No generalizations are apparent regarding compounds that give definite colors. Both thianaphthene and dibenzothiophene produce brown colors reminiscent of thiophene. On the other hand, pure 2- and 3-*tert*-butylthiophene fail to exhibit the chromotropism characteristic of the thiophene nucleus, while 2-*n*-butylthiophene gives the usual test.

Although the above observations appear at first glance to be incompatible, a consideration of the tendency of cerate salts to form molecular complexes (*6*) suggests an explanation. The ability of certain aromatic hydrocarbons, as well as condensed thiophene systems, such as thianaphthene and dibenzothiophene, to form complexes with picric acid is well established. In addition, the bathochromic effect—i.e., the heightening of color—produced by the formation of molecular complexes between polynuclear aromatic hydrocarbons or heterocyclic compounds and picric, styphnic, or picrolonic acids has been observed by nearly all organic chemists. The color bands produced on silica gel during adsorption experiments may also be evidence of the bathochromic effect of a loose molecular complex involved in chemisorption. Thus it appears reasonable to assume that the color reactions of thiophene compounds result from formation of a molecular complex with the cerate salt. The color sequences observed in nearly all the color tests apparently are due to oxidation by the cerate ions. This ultimately causes excessive degradation of the thiophene nuclei, thus making identification of the color complexes impossible.

Although thianaphthene and dibenzothiophene are known to be planar molecules, the *tert*-butylthiophenes are not planar. Thus the anomalous results with the two isomeric *tert*-butylthiophenes and the positive result with the planar 2-*n*-butylthiophene may be explained on the basis of steric hindrance. In support of this conclusion, recent data on the formation of picrates of the benzene series indicate that *tert*-butylbenzene forms the least stable picrate of the entire monoalkyl series (*1*).

The weak color shown by the chlorothiophenes and the ceric nitrate reagent merits comment. 2-Chlorothiophene is much less reactive than thiophene. The relative reactivity of the 5-hydrogen is 0.14 compared to thiophene at unity (*4*). In the benzene

[1] Present address, Experiment Station, Hercules Powder Co., Wilmington, Del.

Table I. Color Reactions of Thiophene Derivatives

Compound	Color
2,3-Dimethylthiophene	Green→green-brown ppt.
2,4-Dimethylthiophene	Red → deep red ppt. → purple organic layer
2,5-Dimethylthiophene	Purple → red → dark purple ppt.
3,4-Dimethylthiophene	Deep blue → deep blue ppt.
2-Ethylthiophene	Red → red ppt.
2-*n*-Propylthiophene	Red → red ppt.
2-*n*-Butylthiophene	Red → red ppt.
2-*tert*-Butylthiophene	Light orange forming very slowly
3-*tert*-Butylthiophene	Light yellow forming very slowly
2,3,4-Trimethylthiophene	Green → brown → purple ppt.
2,3,5-Trimethylthiophene	Purple → brown ppt.
2,3,4,5-Tetramethylthiophene	Blue → red → reddish brown ppt.
Thianaphthene (benzothiophene)	Brown → brown solid
Dibenzothiophene	Brown → brown solid
2-Acetyl-3,4-dimethylthiophene	Red → brown → light red
2-Acetyl-3,5-dimethylthiophene	Red → dark brown → pale yellow
2-Acetyl-4,5-dimethylthiophene	Red → light brown → light red
2-Acetyl-5-ethylthiophene	Cherry red → light brown → light red
2-Acetyl-3,4,5-trimethylthiophene	Purple → deep brown → light red
3-Acetyl-2,5-dimethylthiophene	Pink → light tan → pink
3-Acetyl-2,4,5-trimethylthiophene	Green → brown → dark red

series, introduction of a chlorine atom on the benzene ring decreases the reactivity of that molecule in subsequent substitution reactions. From these observations and from dipole moment studies on chlorothiophenes (*5*), it would appear that the chlorine atom is exhibiting its characteristic as an electron-withdrawing agent. Thus, the electron densities at the carbon atoms of the thiophene nucleus are markedly decreased to a point that the ceric nitrate reagent complexes only slowly or not at all.

In general, the colors produced by reaction of various thiophene derivatives with this reagent are not specific. The 2-alkylthiophenes, where the alkyl group is C_1 to C_4, show almost identical red colors. Oddly enough, each of the nine methylthiophenes, except 3-methyl and 3,4-dimethylthiophenes which produce predominantly blue colors, has a characteristic brilliant color sequence. By observations of the color sequence, it is possible to identify a particular isomer. Mixtures of isomers have not been studied thoroughly, but the expected purple color resulted from a mixture of 2-methylthiophene and 3-methylthiophene, which show red and blue colors in the pure state (*3*).

The homologous acetylmethylthiophenes (see Table I) show interesting color sequences which are not so characteristic either for members of the homologous series or for isomeric forms as are the colors of the isomeric methylthiophenes.

Because of the high intensities of the colors produced by the methylthiophenes, it was of considerable interest to determine whether or not this test might be applied to the detection of thiophene homologs in petroleum distillate fuels. For that reason, it was necessary to study the color reactions of various classes of compounds known to be present in straight-run, thermal, and catalytic distillate fuels. While sulfur compounds other than thiophene fail to produce an interfering color reaction with the reagent, pyrroles, indole, carbazole, and phenol gave extremely sensitive tests and the colors produced appeared to be stronger than for the thiophenes. For example, 3-methylthiophene was found to produce a detectable blue color test in "iso-octane" (2,2,4-trimethylpentane) or benzene in concentrations of 1 part in 200, while 1 part of carbazole in 10,000 parts of benzene gave a detectable emerald green color test.

The only other major class of heterocyclic compounds known to be present in these fuels, that gives no interfering color tests, is the pyridine and quinoline series. The data for all these tests are summarized in Table II.

Of the nonthiophenic sulfur compounds tested, only sulfides give a significant color reaction. The results are readily distinguishable from those with thiophene by three observations: At the beginning of the agitation a definite red color is observed in the aqueous layer; a red organic precipitate forms; and continued agitation for a 2- to 5-minute period produces a colorless aqueous single-phase system. A confirmatory test for sulfides may be made by adding a drop of reagent to the clear solution, whereupon the original reddish orange precipitate forms which undergoes reversion to the water-soluble colorless state. Disulfides and tetrasulfides are quickly converted to a colorless water-soluble phase but do not give this confirmatory test. The water-soluble colorless stage of this reaction appears to have produced a very stable organic intermediate, since addition of reagent over a period of 14 days gives the confirmatory test.

Low concentrations of thiacyclopentane in iso-octane (1 to 20) gave no perceptible color in the organic layer, but the reddening of the aqueous layer took place; this is a positive color test for the sulfide. In such cases, the low concentrations of sulfide employed were not sufficient to produce the last two phases of the test as described above.

The red color of the inorganic layer produced by the sulfides tested is almost identical to that produced by addition of a drop of alcohol to the reagent. Thus, in testing for low concentrations of sulfides, it is necessary to know that alcohols are absent.

TEST PROCEDURES

The test procedure is essentially the same as described in the original communication (*3*). The test solution is prepared by dissolving 400 grams of hexanitroammonium cerate in 1 liter of 2 *N* nitric acid. It has been found to be advantageous to use 0.5 ml. of the ceric nitrate reagent with 0.05 to 0.1 ml. of compound to be tested. Vigorous shaking is essential to produce any color at all with 2- and 3-*tert*-butylthiophene. Color sequences noted in Table I are those observed over a period of 10 minutes. Usually the first two transitions take place in a matter of seconds upon agitation of the test tube, while the final color stage is produced by a few minutes of gentle agitation.

Table II. Miscellaneous Compounds

Compound	Color
2,5-Dimethylfuran	Red oxidizing to colorless solution
2,4-Dimethylpyrrole	Black ppt.
2,5-Dimethylpyrrole	Reddish brown solution
Indole	Greenish brown ppt.
Carbazole	Emerald green ppt.
Pyridine	White ppt.
α-Picoline	White ppt.
Quinoline (synthetic)	Orange ppt.
Phenol	Deep brown organic layer, orange inorganic layer
m-Thiocresol	Light pink
p-Thiocresol	No color change
n-Propanethiol	Slowly dissolves, no color change
n-Butanethiol	Light orange organic layer
Ethanedithiol	White ppt.
n-Butanedithiol	Pinkish white ppt. → red organic layer, colorless inorganic layer
n-Propyl sulfide	Red → dissolves to give colorless solution
Thiacyclopentane	Red → dissolves to give colorless solution
2-Methylthiacyclopentane	Red → dissolves to give colorless solution
Thiacyclohexane	Red → dissolves to give colorless solution
n-Hexyl sulfide	Red ppt. → colorless organic layer
Methyl disulfide	Oxidizes to colorless solution
Ethyl disulfide	Oxidizes to colorless solution
tert-Heptyl disulfide	No color change
Ethyl tetrasulfide	Oxidizes to colorless solution
n-Butyl sulfone	Light orange organic layer, no change in aqueous layer

An attempt to isolate the ceric nitrate-3-methylthiophene color complex led only to formation of brownish black tars insoluble in most common solvents.

SOURCE AND PURITY OF CHEMICALS

Most of the chemicals listed in Table I were prepared in the author's laboratory. Methods of preparation and properties will

be reported elsewhere. All the thiophene derivatives were considered to have a purity of at least 99%.

Samples of 2,3,5-trimethylthiophene and 2,3,4,5-tetramethylthiophene were obtained from F. F. Nord of Fordham University. F. G. Bordwell of Northwestern University supplied samples of thiacyclohexane and 2-methylthiacyclopentane.

The 2- and 3-*tert*-butylthiophene were obtained by precision distillation of a so-called "pure" 2-*tert*-butylthiophene. The "2-*tert*-butylthiophene," "2-*tert*-amylthiophene," and "2,5-di-*tert*-butylthiophene" used originally (*3*) were found to be mixtures, after the disclosure of Appleby *et al.* (*2*) that alkylation of thiophene yielded mixtures of isomers.

Most of the compounds of Table II were Eastman reagent grade products.

ACKNOWLEDGMENT

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Improved Photoelectric Fluorometer

W. C. ALFORD AND J. H. DANIEL

Experimental Biology and Medicine Institute, National Institutes of Health, Bethesda 14, Md.

Rapid expansion of the field of fluorometric analysis has emphasized the need for a photoelectric fluorometer which displays the sensitivity, stability, and flexibility required for quantitative measurements. As no commercially available instrument meets these requirements fully, an instrument was designed. It employs vacuum-type phototubes and an electrometer tube in a circuit which permits measurements of fluorescence, by a null-point method, over an unusually wide range of intensity. The basic fluorometer is powdered by dry cell batteries and is characterized by a very low power consumption, which tends to promote excellent stability. The circuit is such that full advantage may be taken of the ultimate sensitivity of the phototube surface. This fluorometer should permit more accurate analyses by existing methods and facilitate research in the development of new methods.

THE use of fluorescent reagents in analytical chemistry has become increasingly common during the past seventeen years. They were used first as simple qualitative reagents for various inorganic and biological materials, but in recent years many precise quantitative methods have been developed for a wide variety of substances. No attempt is made here to review this field, as two comprehensive reviews of fluorometric analysis have been published recently (*7*, *8*).

Instrumentation has tended to lag behind the development of analytical methods, so that often the worker has been handicapped by lack of an instrument that would meet his needs. Visual comparison methods have been used (*3*), and various, more or less successful, attempts have been made to adapt spectrophotometers to fluorometric measurements (*4*). At present, there are at least ten or twelve instruments on the market which have been designed for photofluorometric work, and several others have been described (*7*, *8*). Practical experience has shown, however, that most of these suffer from one or more of the following objections: lack of stability, inadequate sensitivity, insufficient flexibility to meet widely varying conditions, or excessive cost. The fluorometer described below overcomes these difficulties to a large extent.

DESCRIPTION OF INSTRUMENT

The need for sensitivity and stability dictates the use of a vacuum-type phototube as the light-receiving element. The phototube emissive surface is selected from commercially available types to give greatest response in the spectral region in which fluorescence is expected. For wave lengths above 5000 A., an RCA 919 tube (spectral response S-1) was used; for wave lengths below 5000 A., a Cetron-CE-99 (class Q, spectral response S-4, Continental Electric Co., Geneva, Ill.) was used.

The extremely high impedance and comparative independence of current on voltage of the phototube make it ideally suited to work into an electrometer-tube current amplifier with its associated high input impedance (*2*, *5*). This type of electrical amplification was chosen in preference to an electron multiplier because it appears to be more flexible, reliable, and simple, while taking full advantage of the ultimate sensitivity of the phototube.

The Victoreen electrometer tube VX-41A with its low filament current requirement is well suited to the circuit shown in Figure 1. This is essentially the DuBridge-Brown type circuit (*2*).

The functioning of the circuit may be explained briefly as follows: With a 10-ma. current through filament F, no light incident on phototube T, and the variable taps of potentiometers P_1 and P_2 set at ground potential, P_4 is adjusted to give no deflection of the galvanometer. Current induced by light falling on the phototube then will produce a voltage change (the IR drop across the high resistance connected to S_4) on grid G_2, which causes the electron currents to plate P and grid G_1 to change in opposite directions, thus giving a deflection of the galvanometer. The galvanometer may be returned to zero by applying an equal but opposite voltage change to G_2 by means of P_2 (or P_1). Thus the setting of P_2 becomes a measure of the light intensity on the phototube, with the advantages associated with a null point method of measurement. The function of the remaining components is described below.

STRUCTURAL DETAILS

The instrument described here, and shown in Figure 2, was assembled from equipment that was immediately at hand. Thus, its design is not necessarily that which would provide maximum convenience to the operator. However, it has given trouble-free and entirely satisfactory service for over two years, during which time only one new set of 1.5-volt batteries has been installed.

The various parts of the fluorometer, exclusive of the 1.5-volt batteries, E_1, E_2, and E_3, the ultraviolet source, and the galvanometer are housed in a light-tight wooden box (13 × 13 × 6 inches) which is metal-lined to furnish the necessary electrostatic shielding of the grid lead, G_2, of the electrometer tube. The box is painted inside and out with a nonfluorescent,

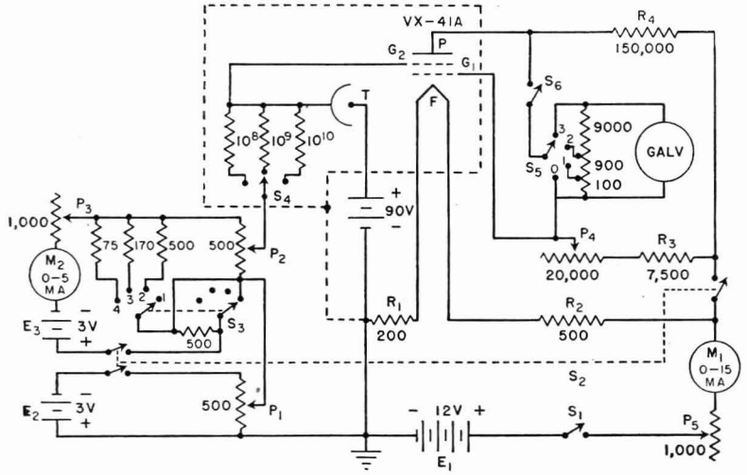

Figure 1. Circuit Diagram of Photoelectric Fluorometer

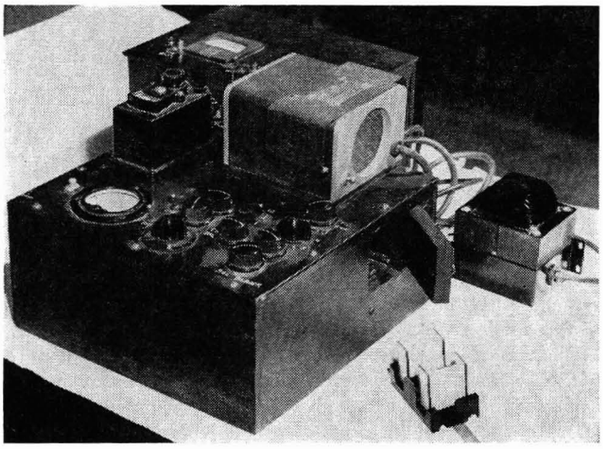

Figure 2. Photoelectric Fluorometer

flat, black paint. A separate wooden box, fitted with appropriate binding posts, holds batteries E_1, E_2, and E_3.

As may be seen in Figure 2, all operating controls are mounted on the metal top of the box. The actual layout of these is unimportant, except that the most frequently used controls (P_1, P_2, and P_3) should be in a convenient location.

The source of ultraviolet radiation is a Coleman Model U-11-M high pressure mercury vapor lamp (AH-4) with constant voltage transformer. In keeping with its design, it is mounted on the top of the instrument at the right rear corner. It is held in place by three pins in the lamp housing, which fit into holes bored in the top of the case. Leakage of light is prevented by a black felt gasket cemented to the under side of the lamp housing. A flat strip of sheet metal (4 × 9 inches) with a hole 1.5 inches square 3 inches from one end serves as a sliding shutter between the lamp and the cuvette compartment. Guide pins in the top of the case and slots in the metal strip assure accurate positioning of the shutter.

The cuvette compartment is located immediately beneath the ultraviolet lamp and measures 4 × 6 inches. It is constructed of wood and Masonite and is completely walled off from the rest of the case, so that no scattered ultraviolet light can reach the phototube. Samples are inserted through a light-tight side door and are held by a two-place cuvette holder (Coleman No. 11-103, Figure 2) which slides on parallel brass rods mounted on a wooden platform of appropriate height. A slot in the base of the cuvette holder and a pin in one of the brass rods permit accurate placement of either cuvette in the light path. Coleman No. 11-121 rectangular fused glass cuvettes (25 ml.) are used.

A cross-sectional diagram of the optical system, as seen from the door of the cuvette compartment, is shown in Figure 3. This sketch is largely self-explanatory but it is desirable to keep the light path as short as possible in order to obtain maximum sensitivity—for example, the phototube should be not more than 1 inch from the cuvette.

The filter holder is made of black sheet brass with a 1-inch-square window in the center. It is spring-loaded, as shown, to hold the filters snugly and at the same time permit easy insertion of any desired filter combination. Felt and metal washers under the springs serve to prevent leakage of light. A shutter is not needed between the cuvette and phototube, as such tubes are not subject to fatigue and the galvanometer is protected against sudden surges of current by microswitch S_6.

Thus, the beam of ultraviolet light falls on the surface of the fluorescent solution from above. The resultant fluorescent light, picked up at right angles to the path of the exciting beam, passes through an appropriate filter system and then strikes the active surface of the phototube.

The basic filter system for most work consists of a Corning glass filter No. 5874, mounted in the lamp housing to isolate the 3657 A. mercury line, and a Corning filter No. 3060 between the cuvette and phototube to stop scattered ultraviolet radiation. Additional filters are installed as needed to isolate the wave length of fluorescent light that is to be measured.

The phototube must be shielded from the light emitted by the filament of the electrometer tube.

The galvanometer used for most work is a small Leeds & Northrup portable pointer type with a sensitivity of 1 microampere per millimeter scale division. A more sensitive galvanometer with variable sensitivity (Figure 1) is advantageous when measuring very low intensity fluorescence. In such case, the galvanometer should preferably be of the high resistance type (500 ohms or more) rather than the low resistance type (25 ohms or less) as is used with thermocouples.

OPERATIONAL PROCEDURE

Relative measurements of fluorescent intensity are obtained by a null point method by measuring in arbitrary scale units the volt-

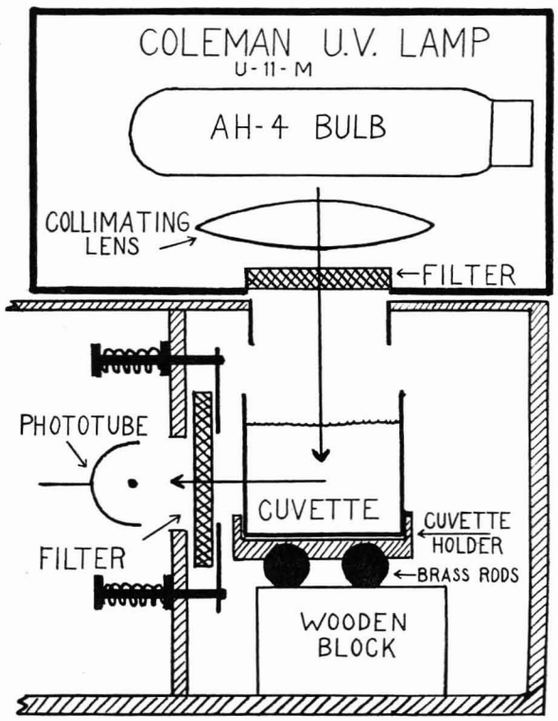

Figure 3. Diagram of Optical System of Fluorometer

age required to balance that produced across a high resistance by the phototube current. This phototube current is, of course, strictly proportional to the intensity of the fluorescent light. The instrument is calibrated in the usual manner—i.e., the fluorescence of a graduated series of known standard solutions is measured to determine the relationship between fluorescent intensity and concentration. Measurement of the fluorescence of a similarly prepared unknown sample then gives directly its concentration.

The ultraviolet lamp is turned on, the filament circuit is closed by means of S_1, and meter M_1 is adjusted to 10 ma. with potentiometer P_5. After a warm-up period of 10 to 15 minutes all other switches (except microswitch S_6) are closed. The shutter between the ultraviolet lamp and the instrument is closed (giving "dark conditions"), and with P_1 and P_2 set at ground potential (zero scale), P_4 is adjusted until no galvanometer deflection is noted when S_6 is closed. Final adjustment is made with S_5 set for maximum galvanometer response (position 3). P_4 is left in this "balanced" position and the instrument is ready for use.

A reagent blank, a standard solution, and an unknown are prepared simultaneously in such manner that they are identical except for concentration of the fluorescent substance. After the appropriate standing period, the reagent blank and standard solution are placed in the cuvette holder, and the blank is positioned in front of the phototube with the fluorometer set at medium sensitivity—i.e., selector switch S_4 set on the 10^8-ohm resistor, and S_3 in the No. 1 position. The galvanometer microswitch, S_6, is closed, and any deflection of the galvanometer is balanced out by adjustment of P_1. P_1 is left in this position and the standard solution is moved into place. The linear potentiometer, P_2, is set at any desired scale reading, say 100, S_6 is closed, and the galvanometer is nulled by adjustment of P_3.

By this procedure, the instrument has been set so that, on P_2, the blank reads zero, and the standard solution reads 100. The standard is replaced by the unknown, and the galvanometer is zeroed by adjustment of P_2. The scale reading of P_2 is now a measure of the concentration of the unknown relative to that of the standard. If, for example, the unknown gives a reading of 70 compared to a standard which reads 100, the unknown contains 70% as much fluorescent material as the standard.

Table I. Test of Fluorometer with Aluminum–Pontochrome Blue Black R Fluorescence

Aluminum, γ/25 Ml.	Scale Readings	
	Run 1	Run 2
0	0	0
2.0	20	21
4.0	39	40
6.0	60	60
8.0	80	81
10.0	100	100

This simple procedure is applicable when it has been found that fluorescent intensity is proportional to concentration (linear calibration curve). If this is not the case, concentration may be determined by reference to appropriate calibration curves. However, because of such variables as temperature, battery strength, ultraviolet light intensity, etc., it is necessary to calibrate the instrument with solutions of known concentration immediately before measuring the fluorescence of an unknown. This technique is required for fluorometric methods in general. The cuvettes must be carefully matched, especially when measuring fluorescence of low intensity. When perfect matching cannot be achieved, the same cuvette must be used for all solutions.

The arbitrary instrument settings of S_3 and S_4 given in the above procedure are applicable only when the fluorescent intensity is in a certain rather narrow range, and are changed to meet varying conditions of concentration and fluorescent intensity. As a given light intensity is measured by balancing the potential drop due to a constant current through the high resistance connected to S_4, the response of the fluorometer to any given light signal is proportional to the magnitude of this resistance. It is thus necessary to select the appropriate resistor and galvanometer sensitivity to give the desired response.

In this respect the instrument offers unusual flexibility. Consideration of the circuit diagram (Figure 1) will show that galvanometer response to a given photo current—i.e., sensitivity—can be varied by a factor of approximately 10,000. This represents the difference between the settings: S_4 on the 10^8-ohm resistor with S_5 in the No. 1 position and S_4 on the 10^{10}-ohm resistor with S_5 in the No. 3 position. Such flexibility is desirable because the fluorescent intensity of different substances varies over a very wide range and also because it allows the operator considerable latitude in selecting a suitable concentration range.

Table II. Relation of Zirconium Concentration to Scale Readings

Curve 1[a]		Curve 2		Curve 3		Curve 4	
ZrO_2[b]	Scale	ZrO_2[b]	Scale	ZrO_2[b]	Scale	ZrO_2[b]	Scale
0.0	0	0.0	0	0.0	0	0.0	0
0.5	8	1.0	9	2.0	10	5.0	11
1.0	19	2.0	20	4.0	19	10.0	21
1.5	29	3.0	31	6.0	30	15.0	29
2.0	39	4.0	40	8.0	38	20.0	40
2.5	51	5.0	48	10.0	49	25.0	51
3.0	60	6.0	60	12.0	61	30.0	61
3.5	71	7.0	70	14.0	71	35.0	70
4.0	80	8.0	79	16.0	80	40.0	79
4.5	92	9.0	90	18.0	90	45.0	89
5.0	100	10.0	100	20.0	100	50.0	100

[a] "Curve" is used here to represent a set of calibration data.
[b] Micrograms per 25 ml. of solution.

Flexibility is also provided by the resistors, which may be paralleled across P_2 by means of S_2 to reduce the potential across P_2, and thus permit setting of the instrument to give any desired scale reading (100 in the example given above) on any appropriate fluorescent standard. Each successive position on S_3 (1 to 4) approximately doubles the scale reading of a given fluorescent solution. The need for such a system is obvious when it is considered that the high resistors, S_4, vary from each other by factors of 10, and that, with S_3 in any given position, the potential across P_2 can be varied only by a factor of roughly 3 by adjustment of P_3. This ability to set the instrument to give a large scale reading for any given fluorescence serves to increase the percentage accuracy of scale readings and is a convenience to the operator in establishing calibration curves.

REPRESENTATIVE DATA

The completed instrument was first tested by using it for the determination of aluminum by the Pontochrome Blue Black R method under the optimum conditions given by Weissler and White (*6*). Typical calibration data are shown in Table I.

The instrument was also used in the development of a new fluorometric method for zirconium (*1*). Table II gives the results obtained on a series of calibration runs covering different ranges of zirconium concentration. This table illustrates one of the desirable features of the instrument—that for any usable concentration range (of fluorescent material) the percentage accuracy of a particular scale reading is constant. For example, the percentage accuracy in the determination of 10 micrograms of zirconium dioxide (scale reading 21 of curve 4) is the same as that of the 1-microgram sample (scale reading 19, curve 1). It is also apparent that the absolute error is approximately constant over the entire range of scale readings, although percentage accuracy is best at the higher scale readings.

Measurements were also made on fluorescein and quinine sulfate solutions, and in both cases scale readings were a linear function of concentration.

In order to demonstrate the sensitivity and range of the fluorometer, solutions of quinine sulfate were prepared, in 0.1 *N* sulfuric acid, to contain, respectively, 20, 10, 1, 0.1, 0.01, and

0.001 microgram of the salt per milliliter. A Wratten C-5 gelatin filter was used to isolate the blue fluorescent light of the quinine solutions (between cuvette and phototube). Twenty-five milliliters of each solution were measured in the fluorometer, using 25 ml. of 0.1 N sulfuric acid as a reagent blank in each case. The galvanometer used had a sensitivity of 0.05 microampere per millimeter scale division.

Results are shown in Table III. Instrumental settings are given to illustrate the various combinations which may be employed to permit measurement of a given fluorescent solution.

DISCUSSION

Assuming a judicious choice of light filters, and the absence of leakage currents (discussed below), the accuracy of measurements depends primarily on the linearity of potentiometer P_2 (General Radio Type 301-A is satisfactory) and the stability of the ultraviolet source. Stability of the electronic amplifier becomes a factor only if a very sensitive galvanometer is used for measuring extremely weak fluorescence. This type of instability can be minimized as follows:

As the filament current is varied between 9 and 13 ma. (rebalancing with P_4 if the galvanometer goes off-scale), the galvanometer deflection will pass through a maximum (or minimum). The filament current is then set at the value giving this maximum —i.e., where the rate of change of deflection with filament current is zero—and the galvanometer zeroed with P_4. If this filament current is not in the range 9.5 to 11.0 ma., values of R_2 in the range 400 to 600 ohms, and, if necessary, of R_1 in the range 150 to 400 ohms can be tried until the deflection maximum (or minimum) does occur at a filament current in the neighborhood of the rated value of 10 ma. With the tube used, and the values of R_1 and R_2 shown in Figure 1, this "compensated" condition was obtained at a filament current of 10.0 ma. However, for the range of fluorescent intensities normally encountered, the fluorometer was used "uncompensated" with R_1 equal to 300 ohms and R_2 equal to 450 ohms (as recommended for electrometer use by the manufacturer).

Should it be found impossible to zero the galvanometer with P_4, a different value of R_3 is called for.

As a safeguard against current leakage across the phototube, a grounded guard ring is provided. This consists of several turns of fine, bare wire wound around the glass envelope near its base and coated with Aquadag to ensure good electrical contact to the glass. The surface of the glass envelope between guard ring and cathode is kept clean and free of dirt and grease.

To reduce leakage current from G_2 to ground, the grid lead from phototube to amplifier is made as short as possible and is not allowed to contact any other wire or surface. Leakage across the surfaces of the high resistances connected to S_4 is minimized by the use of Victoreen vacuum-sealed Hi Meg resistors. The contacts of S_4 should have an insulation resistance higher than 10^{10} ohms. Of the common types of radio selector switches, those having ceramic insulation probably meet this requirement best (Mallory 161C Hamband is excellent).

With such precautions, leakage currents have caused trouble only under extremely humid conditions, and it has not been necessary to use a desiccant in the fluorometer case under normal weather conditions. Troublesome leakage currents are evidenced by (1) a decreased response of the galvanometer to changes in P_1 or P_2 for the higher resistances of S_4, and (2) for a given light signal, a lack of proportionality of galvanometer response or nulling voltage to the value of the resistor connected to S_4—i.e., the response or nulling voltage for the 10^{10}-ohm resistor will be less than ten times that for the 10^9-ohm resistor. If such symptoms indicate the need for a desiccant, a nonliquefying type (such as silica gel or calcium sulfate, rather than calcium chloride or phosphorus pentoxide) should be used.

Stability in an instrument of this kind is an easily observed quality, but one that is not readily demonstrated with experimental data. However, at any low or medium-sensitivity setting the instrument described above is characterized by an almost complete absence of "drift" under "dark" conditions and at highest sensitivity the need for rebalancing seldom occurs. The data given in Tables I and II were obtained with no rebalancing of the circuit after the initial settings had been made. That stability is to be expected is evident when it is considered that the electrometer tube is operated with grid resistors considerably smaller than those which can be used with this tube. The manufacturers state that the tube itself has an external leakage resistance of 10^{15} ohms and that resistances up to 10^{12} ohms can be successfully used in circuits of the type described.

Table III. Sensitivity and Range of Fluorometer

(For quinine sulfate solutions)

Quinine Sulfate, γ/25 Ml.	Grid Resistor, Ohms, S_4	Position of S_5	Position of S_3	% of P_3 in Series with P_2	Scale Reading
0.025	10^{10}	3	4	100	7
0.25	10^{10}	3	4	100	63
2.5	10^{10}	3	1	100	59
2.5	10^9	3	1	100	6
2.5	10^9	3	3	100	22
25.0	10^9	3	1	100	58
25.0	10^9	3	2	80 (approx.)	100
250.0	10^8	2	2	0	39
500.0	10^8	1	1	0	46

Under normal working conditions, any lack of reproducibility of measurements can be attributed mostly to the ultraviolet source. The intensity of the exciting radiation varies with line voltage and the constant voltage regulators usually employed do not provide perfect control. Changing battery strength over any short period of time is negligible and, in fact, the operating life of the batteries used in this circuit may be expected to approach their shelf life.

SUMMARY

A new photofluorometer, employing a vacuum-type phototube and a Victoreeen electrometer tube, has been developed. Measurements are made by a null point method in which the fluorescence of an unknown solution is compared to that of a standard solution. The design of the instrument offers definite advantages of stability, sensitivity, flexibility, accuracy, and convenience of operation over commercially available instruments. The instrument as described may be readily constructed at a material cost considerably below that of any commercially available fluorometer. Current price lists indicate a total parts cost of slightly over $100, exclusive of ultraviolet lamp and galvanometer.

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Magnetic Mercury Cathode

E. J. CENTER, R. C. OVERBECK, AND D. L. CHASE
Battelle Memorial Institute, Columbus, Ohio

This study was undertaken to develop a more practical mercury cathode for use in analytical separations. A new mercury cathode has been designed which employs a magnetic circuit. The cathode removes metals rapidly and completely, is rugged and convenient to operate, and contains no mechanical moving parts. The novel magnetic circuit provides rapid countercurrent stirring at the mercury electrolyte interface, continuously cleans mercury surface (ferromagnetic element), and requires a minimum amount of mercury per analysis. Electrolysis with the mercury cathode is often used as a means of separation of certain elements in analytical techniques. It is particularly useful where large amounts of interfering metals must be separated from small quantities of other elements to be assayed. The new cathode provides the analytical chemist with a practical, rapid tool for quantitative analytical separations.

THE mercury-cathode electrolysis is often one of the most reliable, if not the only practical, means for the separation of certain elements. Because of the large number of elements deposited and the inconvenience of weighing the mercury, the cathode is usually employed for separation rather than direct determination. The technique is particularly useful where large amounts of interfering metals must be separated from small quantities of other elements which are not deposited.

In dilute sulfuric acid solution, elements such as aluminum, titanium, zirconium, vanadium, and uranium can be quantitatively separated from iron, nickel, cobalt, copper, and other elements of the mercury-cathode group (*7*). In general, the electrolysis has been carried out in a glass cell with a mercury-pool cathode and a platinum anode. Numerous cathode cells employing a variety of stirring mechanisms, electrode designs, current densities, heat exchangers, etc., have been described in the technical literature (*1, 2, 4, 6, 8–11*). However, the mercury cathode has never been fully utilized as an analytical tool, because of low rates of deposition; lack of quantitative separation in certain systems due to instrument and cell design; difficulties in the handling of the electrolyte, mercury, and amalgam during and after electrolysis; or high initial and operating cost of equipment.

The following work was undertaken in an attempt to develop a more practical mercury cathode.

PRELIMINARY STUDIES

To investigate the factors affecting the removal of metals from solution, two identical cells, 13 cm. deep and 6.5 cm. in diameter, were made of borosilicate glass. A cooling jacket, 1 cm. thick, surrounded each cell from the top to within 1 cm. of the bottom (Figure 1). In this type of cell, the electrolyte was withdrawn from the top of the mercury, thus avoiding the necessity of attempting to force the mercury amalgam through a stopcock upon completion of electrolysis.

The cathode consisted of approximately 50 sq. cm. (35 ml.) of mercury. A platinum wire, B. & S. gage No. 17, weighing about 16 grams, was formed into a flat spiral (Figure 1) to serve as the anode.

Because the removal of iron and paramagnetic metals is desired in about 95% of all analyses using the mercury cathode, the magnetic properties of these metals were utilized to assist in electrolysis. Thus, a large, horseshoe-type magnet of Alnico V was placed under one of the cells, as shown in Figure 1. The second cell was used for control to compare with the over-all effect of the magnet. To obtain comparable results, the two cells were placed in series with an ammeter and a variable resistance, and connected to a direct current power supply.

By placing the magnet so that one pole was below the center of the cell and the other pole at the outer edge of the cell, two novel and most desirable effects were produced. The iron amalgam was pulled below the mercury surface as rapidly as it was produced; thus, re-solution of the iron was completely prevented. The strong magnetic field caused the electrolyte to rotate when current flowed through the cell. The electrolyte behaved like a simple conductor in a magnetic field and acted as the rotor in a simple direct current motor. The speed of the rotation depended on the amount of current flowing and the strength of the magnetic field. Frary has described the use of a solenoid for stirring solutions during electroanalysis (*5*).

A number of electrolyses were performed with iron, nickel, cobalt, copper, chromium, and zinc in sulfuric acid electrolyte under identical conditions with the magnetized and the unmagnetized cells. Currents of 5 to 10 amperes were used. In both cells an increase in temperature resulted in a somewhat higher rate of metal deposition. Optimum geometry of the anode and the cathode was essentially as shown in Figure 1 for normal cathode operations; the area of the anode was critical up to 28 sq. cm., but a larger anode showed no increase in efficiency. The volume of the electrolyte should be kept as small as practical (the removal of 1.0 gram of iron from 50 ml. was accomplished in

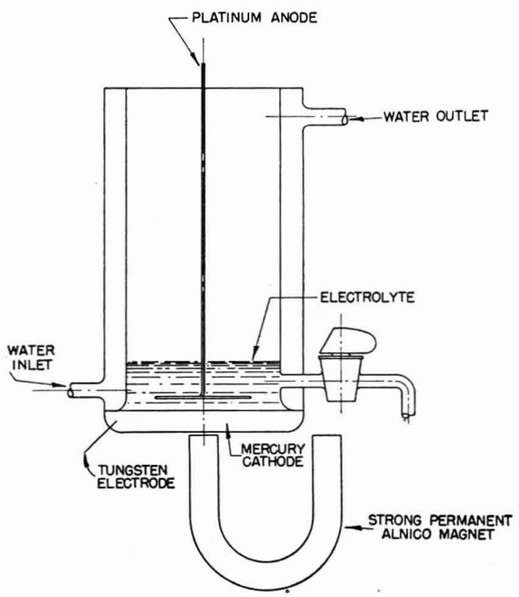

Figure 1. Experimental Model of Mercury Cathode Cell

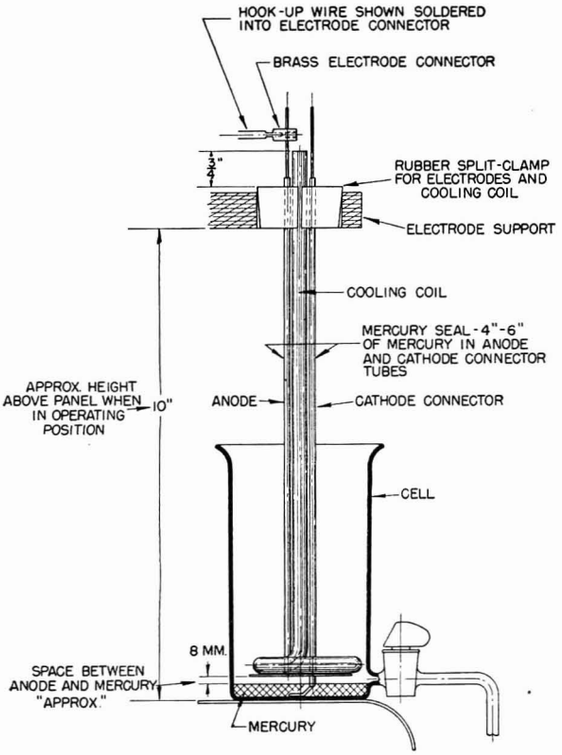

Figure 2. Mercury Cathode Cell

Elevation showing proper location of parts

about half the time required to remove the same amount from 100 ml.). "Ripple-free" direct current was not essential for efficient operation of the cells.

Optimum deposition rates were obtained with an acid concentration (sulfuric acid) from 0.1 *N* to 0.5 *N*, although much higher acidities could be maintained in the magnetized cell because of the minimized re-solution effect.

The deposition of 1.0 gram of iron on a mercury surface of about 50 sq. cm. produced a crust of amalgam in the unmagnetized cell. In the magnetized cell, the cathode was clean and silvery after 10.0 grams of iron had been deposited in 35 ml. of mercury.

The magnetized cell showed consistent advantage in speed and efficiency of removal of both paramagnetic and diamagnetic metals. The advantage was most marked in the tests with cobalt, where the use of the specially placed magnet (Figure 1) cut the time of deposition in half.

MERCURY-CATHODE DESIGN

Cells. The final design of the mercury-cathode cell is shown in Figure 2.

The cell is constructed of borosilicate glass and has a capacity of 400 to 450 ml. A special stopcock is welded near the bottom of the cell so that, when 35 to 50 ml. of mercury are added, the mercury level will be flush with the stopcock outlet. This arrangement permits all but a few milliliters of electrolyte to drain from the cell when the stopcock is opened. Rapid quantitative removal of the cell contents is easily accomplished with a small volume of wash water. The cell is washed in position and the mercury is not disturbed.

Electrodes and Cooling Unit. The anode, cathode contact, and cooling coil are combined to form a probe unit (Figure 2). The anode is fabricated from heavy (B. & S. gage No. 17) sand-blasted platinum wire in the form of a flat spiral. Platinum is also used as a cathode contact to the mercury. Graded seals of No. 3320 uranium glass to borosilicate glass are used in the construction of the platinum anode and cathode connectors. The heat exchanger consists of a single coil of borosilicate glass. The probe assembly can be quickly dismantled and reassembled in event of breakage, adjustment, or, if desired, use of a special electrode. Split watch glasses of plastic cover the cell and probe unit and effectively prevent spray losses.

Magnetic Circuit. Horseshoe-type Alnico permanent magnets are placed under each cell of the cathode unit. The geometry of the magnet and the cell is the same as that shown in the trial cell in Figure 1.

General Construction. The completely assembled magnetic mercury cathode is shown in Figure 3. The unit contains no moving mechanical parts; only the electrolyte and the mercury are in motion during electrolysis.

The case is constructed of heavy-gage, corrosion-resistant stainless steel and cast aluminum with black wrinkle finish. The mercury cathode is 16.5 inches wide, 14 inches deep, and 21.5 inches (over-all) high, with probe assemblies in operating position. The probe assemblies can be raised a maximum of 5.5 inches. The sloping instrument panel carries a cell-selector switch for operation of right or left cell only, or both cells simultaneously; an ammeter reading 0 to 25 amperes showing direct current input to cell or cells; a fuse held in a panel receptacle which is mounted in the direct current circuit and protects the electrical components; a pilot light indicating power to unit (on or off); and a variable autotransformer knob controlling direct current input to cell or cells.

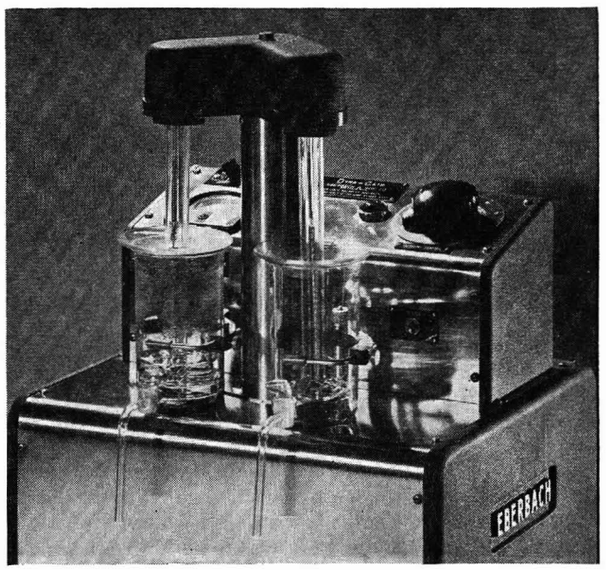

Figure 3. Magnetic Mercury Cathode

The vertical panel has a needle valve controlling the volume of water flowing through the heat exchangers in the cells, and an off-on toggle switch controlling alternating current power into the unit from line source (Figure 4).

The stainless steel pillar in the center of the mercury cathode carries the probe support, which can be raised 5.5 inches when the operator desires to remove the cells for cleaning. Electrical and heat-exchanger leads pass through the pillar and probe support to each of the probe assemblies. The probe support is made of stainless steel and bakelite with a black wrinkle-finished aluminum cap covering the connections in the head of the probe support.

The two cathode cells are held in place with a stainless steel and Bakelite clamp which provides positive centering of the probe unit with the cell. There is complete visibility of the electrolyte, mercury, and control panel in all operations.

OPERATION

The magnetic mercury cathode is a completely self-contained, dual unit operating directly from 115-volt, 50- to 60-cycle alternating current. It needs a source of cooling water, although

this is not required for all operations. The unit is built for continuous laboratory operation with a maximum power requirement of 400 watts. Stepless current control provides 0 to 20 amperes for either or both cells.

By introducing the cathode connector into the center of the mercury (Figure 2), the change in direction of current flow causes the mercury to rotate in the same manner as the electrolyte, but in the opposite direction. Thus, the mercury and the electrolyte become independent rotors of a simple direct-current motor, which results in countercurrent stirring at the interface of the mercury and the electrolyte.

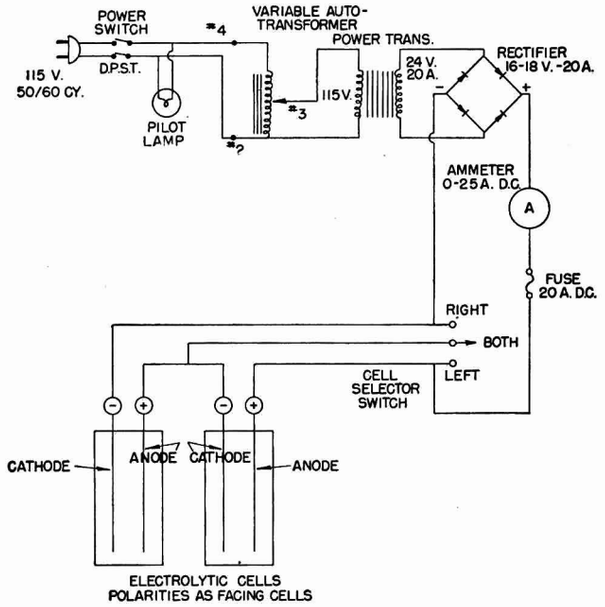

Figure 4. Schematic Wiring Diagram

The detailed mechanism of countercurrent stirring is shown in Figures 4 and 5. Figure 5 graphically illustrates the flow of current through the mercury-cathode cell. Figure 6 shows magnet, cell, and cathode probe location along with flux, current, and force vectors for various positions in the electrolyte and mercury. Magnetic lines of force are assumed to be essentially vertical at *B* and *C* and approaching horizontal at *A* and *D* (the force at *A* will be greater than the force at *D*). The counter rotation of the mercury is confined to the area adjacent to the cathode connector probe. The over-all effect is shown in Figure 7. The countercurrent agitation is independent of the electrolyte or the metals being removed.

In addition, the magnetic field immediately removes deposited ferromagnetic metals from the working interface and retains them beneath the surface of the mercury. Thus, electrolysis is complete and re-solution effects are negligible.

Temperature is controlled when necessary by adjusting the flow of cooling water to the heat exchangers.

Probes and cells can be rinsed and new electrolyte inserted without raising the probe support. The probe support can be raised by loosening the knurled-head setscrew, lifting the probe support, and resetting the screw.

It is not always necessary to change mercury during an electrolysis. Ten or more grams of iron may be deposited in one 40-ml. charge of mercury, although a larger 70-ml. mercury cell may be more convenient for 10-gram samples. On completion of electrolysis, only the electrolyte is drained from the cell through the stopcock. This avoids the troublesome operation (and resulting contamination of the electrolyte) of attempting to force mercury plus amalgam through a stopcock.

The vertical distance between the platinum anode and the mercury may be easily adjusted by opening the split rubber stopper at the top of the probe assembly. For general work, a separation of 6 to 8 mm. is optimum; however, with large amounts of electrolyte containing high concentrations of cathode-group metals, greater spacing may be used.

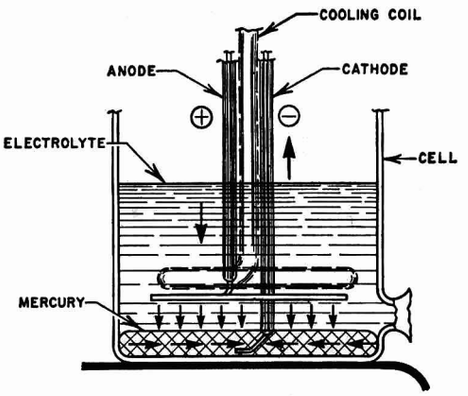

Figure 5. Flow of Current Through Mercury Cathode Cell

To Operate Magnetic Mercury Cathode. Raise probe units and place a cathode cell in each position.

Lower probes carefully to see that the cathode lead just clears the bottom of the cell (adjust by removing the housing over the probe units and loosening the probe holder).

Pour mercury into the cells until the level is just flush with the lower edge of the cell drain and then add the electrolyte.

Place the split plastic cover glasses over the cells.

With power control at the low end of its scale, and the cell-selector switch in the desired position, turn on the main switch.

Adjust power setting to the required amperage (as the resistance of the cell decreases as the electrolysis proceeds, the initial setting should be low enough or a readjustment made later to prevent exceeding 20 amperes). (Occasionally during the electrolysis of concentrated iron solution in excess of 5 grams and at very high current densities, trees may form on the surface of the mercury. They increase the effective area of the cathode

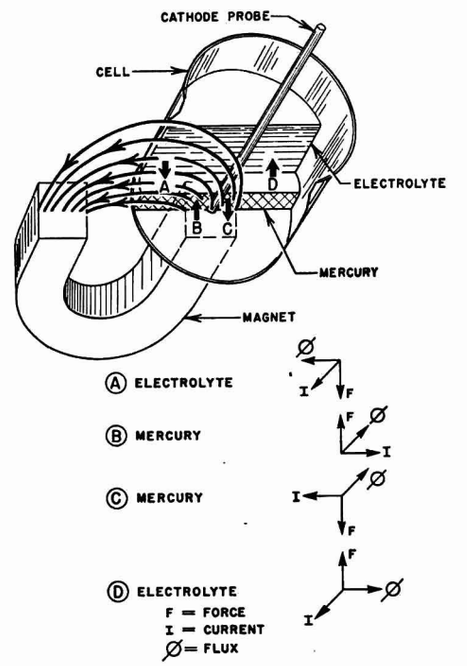

Figure 6. Vector Diagrams Illustrating Countercurrent Rotation in Cell

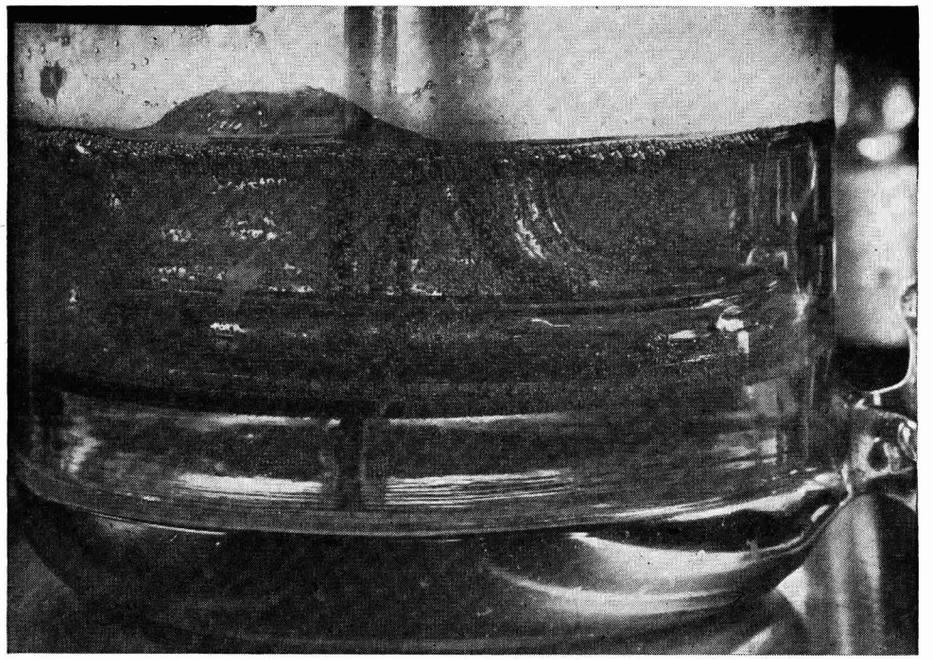

Figure 7. Stirring Action in Magnetic Mercury Cathode

and thus aid in deposition to some extent. As the trees disappear at the completion of electrolysis, they are of no importance unless they grow sufficiently to short-circuit the cell. Should this occur, it can be remedied by increasing the electrode spacing by raising the probe unit slightly, or avoided entirely by using a larger cell.)

Turn on and adjust cooling water, if necessary, by means of the needle valve on the front panel.

Table I. Performance of Magnetic Mercury Cathode

Electrolyte, H_2SO_4, 0.1 N, Ml.	Metal	Grams	Time of Removal by Magnetic Mercury Cathode, Minutes[a]
50	Fe^{+++}	0.5	10 to 15
50	Fe^{+++}	1.0	15 to 20
100	Fe^{+++}	1.0	20 to 35
100	Fe^{+++}	3.0	Less than 65
100	Fe^{+++}	5.0	Less than 90
150	Fe^{++}	5.0	55 to 65
50	Cu^{++}	1.0	7 to 10
100	Cu^{++}	1.0	10 to 15
50	Ni^{++}	1.0	7 to 10
100	Ni^{++}	1.0	10 to 12
100	Zn^{++}	1.0	16 to 17
50	Co^{++}	1.0	10 to 12
100	Co^{++}	1.0	13 to 17
100 (0.5 N $HClO_4$)	Pb^{++}	1.0	Less than 30

[a] Based on final electrolyte content of less than 70 micrograms of metal per 100 ml. of solution. Electrolysis conducted at 15 to 20 amperes with 40 ml. of mercury (cathode area approximately 45 sq. cm.).

At the completion of electrolysis, drain electrolyte from the cell and wash as required. In cases requiring a minimum of cathode-group metals remaining in the electrolyte—i.e., less than 50 micrograms per 100 ml.—wash with the current on. This is easily accomplished by draining the electrolyte to a level just above the anode, adding a small amount of water (or 0.1 N acid), and again draining to the same level. This technique is particularly important with metals that are not ferromagnetic.

PERFORMANCE

Data on the removal of various elements by electrolysis on the magnetic mercury cathode are given in Table I.

Manganese is incompletely separated from the electrolyte, when the mercury cathode is used under normal conditions (*7*). Some of the manganese is deposited on the anode as the hydrated dioxide and some is deposited in the mercury. Table II shows the behavior of manganese using the magnetic mercury cathode. The chemical technique employed is essentially that used by Clopin (*3*). During the electrolysis (Table II) 30% hydrogen peroxide was added dropwise to the electrolyte to remove the dioxide from the anode. Manganese removal is approximately 99.6% complete under the conditions given.

The iron remaining after a standard sodium bicarbonate separation (determination of residual aluminum in a 5-gram sample of plain carbon steel) can be removed on the magnetic mercury cathode in 15 minutes.

Table II. Deposition of Manganese Using Magnetic Mercury Cathode[a]

Experiment	0.1 N H_2SO_4, Ml.	1:5 H_3PO_4, Ml.	4% K_2HPO_4, Ml.	Time, Min.	Manganese Remaining, Mg.
1	95	5	...	30	4.0
2	10 (+71 ml. H_2O)	4 (concd.)	15	30	7.5
3	85	...	15	30	4.0
4	80	5	15	30	3.4
5	75	10	15	30	4.0
6	80	5	15	45	4.6
7	80	5	15	60	4.7
8	80	5 (concd.)	15	45	4.9
9	84	1 (concd.)	15	120	4.2

[a] Starting solution for each experiment contained 1 gram of manganese; electrolysis was conducted at 15 amperes and cell temperature was maintained from 50° to 60° C. by means of cooling coil.

SUMMARY

Major factors affecting the removal of metals from acid solution by means of the mercury cathode have been determined. A magnetic mercury cathode has been developed which removes metals rapidly and completely, is rugged in construction and easy to operate, and contains no mechanical moving parts (only the electrolyte and the mercury are in motion).

The use of the novel magnetic circuit provides rapid countercurrent stirring at the mercury-electrolyte interface, continuously clean mercury surface (ferromagnetic elements), minimum resolution of deposited metals, and a minimum amount of mercury per analysis.

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Rapid Estimation of Small Amounts of Ethylene in Air

Portable Instrument

FRED STITT, A. H. TJENSVOLD, AND YOSHIO TOMIMATSU
Western Regional Research Laboratory, Albany, Calif.

A simple, reliable field method for estimating 5 to 200 p.p.m. of ethylene in air is needed in the citrus industry to avoid degrading of oranges due to overtreatment with ethylene. The portable instrument developed by Beckman, McCullough, and Crane for the microdetermination of carbon monoxide in air has been modified for this purpose. A fixed volume of gaseous sample is passed successively over granular red mercuric oxide at 285° C. and a strip of sensitized paper at 125° C. Mercury vapor formed by the oxidation of the ethylene reacts with red selenium deposited in the paper strip to form a black coloration, the length of which is directly proportional to the ethylene content of the sample. Determinations may be made within a few minutes with an error of less than 10%. Most volatile constituents of fruits likely to interfere can be removed by passing the sample over pumice saturated with 95% sulfuric acid. The instrument may be suitable for checking the adequacy of ventilation in the storage of products which evolve ethylene, such as apples and pears.

ETHYLENE gas is used at low concentrations (10 to 200 parts per million of air) to reduce the chlorophyll content (*18*) in the rinds of oranges which at the time of picking do not have the reddish orange hue usually associated by the purchaser with mature ripe fruit. In addition to its effect on color, ethylene stimulates the respiratory activity of citrus fruits (*9*). Overtreatment with ethylene thus results in poor keeping quality and also destroys the bloom characteristic of prime fresh fruit. Considerable economic loss occurs at present due to degrading of fruit which has been overtreated with ethylene. One reason for this has been lack of a reliable field method for estimating small amounts of ethylene in air.

The object of this investigation was to provide such a method using easily portable apparatus and requiring no special skill on the part of the operator. In addition to its use in controlling treatment with ethylene for the purpose of coloring or ripening, such a method may also be suitable for checking the adequacy of ventilation in the storage of products which evolve ethylene, such as apples and pears (*6*, *16*).

The requirements of simply operated, portable apparatus and sensitivity in the range of 10 to 200 p.p.m. limit the choice of methods available. The experience of Latimer, Ruben, Norris, and Gwinn (*14*) with the use of hot wire heat of combustion type apparatus made this approach unattractive. Attempts to utilize the heat of combustion by catalyzing the oxidation of ethylene with Hopcalite (*13*) were unsuccessful. Likewise, the National Bureau of Standards colorimetric indicating gel method for the determination of carbon monoxide in air (*25*) was found not suitable for ethylene. None of the methods (*6*, *8*, *10*, *11*, *19–22*, *29*) that have been used for measuring ethylene produced by fruits or vegetables seemed adaptable as a simple field method. Recently Young (*29*) has developed a method which is rather specific for ethylene and is suitable for the range of concentration of interest, but which the authors were unable to modify for use as a rapid field method.

About the time this work was begun, A. O. Beckman called attention to a portable instrument which had been developed under a contract with the Office of Scientific Research and Development for the determination of small amounts of carbon monoxide in air (*1*, *2*). The instrument employs hot red mercuric oxide as an oxidizing agent and paper sensitized with selenium sulfide for measuring the amount of mercury vapor formed. The authors have modified this instrument for use in estimating ethylene in air. Although the resulting instrument also responds to oxidizable substances other than ethylene, many of these interfering substances are easily removed before passage of the sample through the instrument.

REACTION OF ETHYLENE WITH RED MERCURIC OXIDE

Temperature. Complete oxidation of ethylene by mercuric oxide would yield 6 moles of mercury per mole of ethylene in accord with the following equation:

$$C_2H_4 + 6HgO \longrightarrow 2CO_2 + 2H_2O + 6Hg \qquad (1)$$

The completeness of reaction of ethylene with mercuric oxide as a function of temperature was followed gravimetrically, using apparatus and technique differing in minor details from that used by McCullough, Crane, and Beckman (*2*, *15*). The results in Table I indicate that for an average contact time of 5 seconds, reaction is essentially complete at temperatures above 275° C. The data of Table I were obtained with standard samples of ethylene in nitrogen. When standard samples of ethylene in oxygen or in air were used, results qualitatively similar to those of Table I were obtained, but a molar ratio of 5.4 was the limiting value attained at temperatures from 275° to 350°. This value was reproducible. Thus the average value of the ratio in fifteen experiments at 300° was 5.37 ± 0.07 mean deviation, and in four experiments at 350°, 5.38 ± 0.01. This difference in completeness of reaction for oxygen and nitrogen atmospheres was also confirmed in later measurements using sensitized paper for following the reaction. The mechanism of oxidation of ethylene by mercuric oxide is apparently different in the presence and absence of oxygen, but the authors have no satisfactory explanation for the maximum value of the molar ratio of 5.4 observed in the presence of oxygen.

Table I. Completeness of Reaction Between Ethylene and Red Mercuric Oxide as Function of Temperature

Ethylene concentration approximately 400 p.p.m. of nitrogen. Average contact time 5 seconds)

Temperature, ° C.	Moles Hg / Mole C_2H_4
150	0.10
200	0.77
225	2.45
250	5.50
275	5.85
285	5.95
300	5.90
350	5.85

Contact Time. Complete reaction may be attained at lower temperatures than 275° by increasing the time of contact of the sample with the mercuric oxide. Thus Table II shows the effect of contact time on the completeness of reaction at 225° C.

Table II. Completeness of Reaction Between Ethylene and Red Mercuric Oxide as a Function of Contact Time at 225° C.

(Ethylene concentration approximately 400 p.p.m. of nitrogen)

Contact Time, Seconds	Moles Hg / Mole C_2H_4
2.5	1.7
5	2.4
10	4.0
19	5.2
75	5.8

Experimental Details. Gravimetric experiments were made with mercuric oxide reaction tubes, 12 mm. in inside diameter and 12 cm. long, provided with ground-glass joints and caps at each end. The reaction tubes were capped when they were not in the cylindrical oven, thus eliminating the necessity of weighing at a specified time after removal of the tube from the oven. Weighings were made on an analytical balance, using an empty tube as tare with reproducibility of 0.1 mg. Standard samples were made, using a gas pipet and gasometer (*2*, *15*). Very poor reproducibility in early experiments was traced to the presence of small amounts of mold which was found growing in the water used as confining liquid in the gasometer. Apparently the mold produced a gas oxidizable by hot mercuric oxide. The mold was not identified, but certain molds are known to produce ethylene (*3*, *17*). Saturated sodium chloride solution was thereafter used as confining liquid in order also to reduce the solubility of ethylene. Most of the gravimetric results were obtained with a gasometer of 3.5-liter capacity by using the entire gaseous content of the gasometer for each run. The gas was usually not dried before entering the reaction tube, as drying was found to have no effect on the results. Blanks were run using large volumes measured by flow rate and time of flow.

Blanks. Commercial tank oxygen, water-pumped tank nitrogen, and laboratory compressed air passed over activated charcoal were used in preparing standard ethylene samples for gravimetric experiments. Blank gravimetric runs on these gases showed little variation with temperature from 225° to 300° C. and gave weight losses small compared to those expected if equilibrium dissociation pressure of the mercuric oxide (*2*) is maintained in the reaction tube at all times. Thus at 285° the expected weight losses for nitrogen and oxygen are 60 and 3.5 mg. per liter, whereas the observed values were 0.20 and 0.03 mg. per liter, respectively. Most of these observed losses were later found to be due to oxidizable impurities in these gases, a finding which is compatible with the small temperature dependence of the observed blanks.

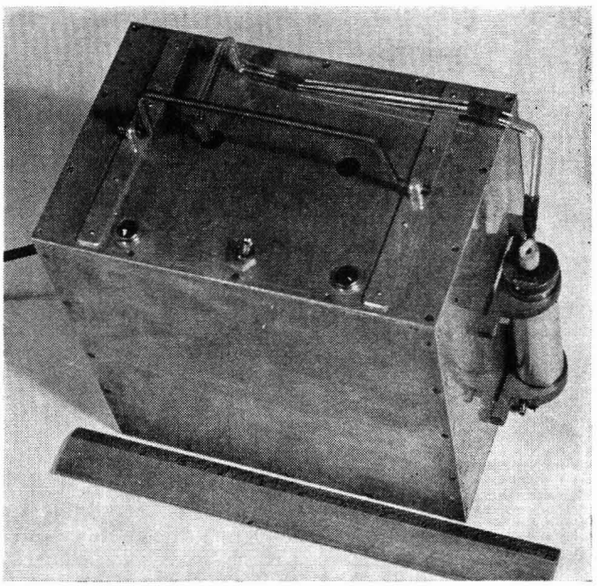

Figure 1. Experimental Model of Ethylene Instrument

High results were invariably obtained for a blank run immediately following an ethylene sample run on the same reaction tube. Likewise the loss in weight was always low for an ethylene sample run immediately after a blank run on the same reaction tube. Similar results indicating a lag in response to new conditions were regularly obtained in later work, in which paper sensitized to mercury vapor was used for following the reaction. This behavior suggests that either mercury vapor is adsorbed by the hot mercuric oxide or that ethylene undergoes sorption followed by relatively slow reaction in the course of its oxidation by mercuric oxide. That sorption of mercury vapor by the mercuric oxide is probably the correct explanation is shown by the fact that similar lags in response were observed under the following conditions, in which ethylene or other oxidizable impurities could not have been present. After repeated measurements with sensitized paper on 38-cc. samples of purified oxygen passed over mercuric oxide at 285° had shown a low reproducible blank value, the reaction tube was allowed to stand for a period of hours, during which equilibrium between the oxide, oxygen, and mercury vapor was approached. Further measurements on successive 38-cc. samples of purified oxygen then showed a lag in response to the new conditions, as indicated by the gradual approach to the original blank value. Thus, the lengths of blackening in one such experiment were: original blank, 1.9 mm.; after standing 44 hours, 32.5, 21.3, 10.1, 4.4, 2.4, 2.2, and 1.9 mm.

PORTABLE INSTRUMENT

Design. The data of Table I indicate that the temperature of the mercuric oxide reaction tube should be kept above 275° C. for maximum reaction and for elimination of errors due to small temperature fluctuations for contact times of the order of a few seconds. Paper sensitized with selenium or selenium sulfide darkened within a few minutes when held at temperatures above 275°. A study of the properties of papers suitable for estimating

mercury vapor (*26*) indicated that the sensitized paper should be held at a temperature above 65° for maximum reactivity. In order to ensure keeping in vapor form the mercury produced by oxidation of a sample containing 200 p.p.m. of ethylene in air, a paper temperature of 125° was chosen. The portable instrument described by Beckman, McCullough, and Crane (*1*, *2*) was accordingly modified so as to provide for separate regulation of the temperatures of the mercuric oxide and the sensitized paper at 285° and 125° C., respectively. A photograph of the experimental model of such an instrument is shown in Figure 1 and a diagrammatic sketch in Figure 2.

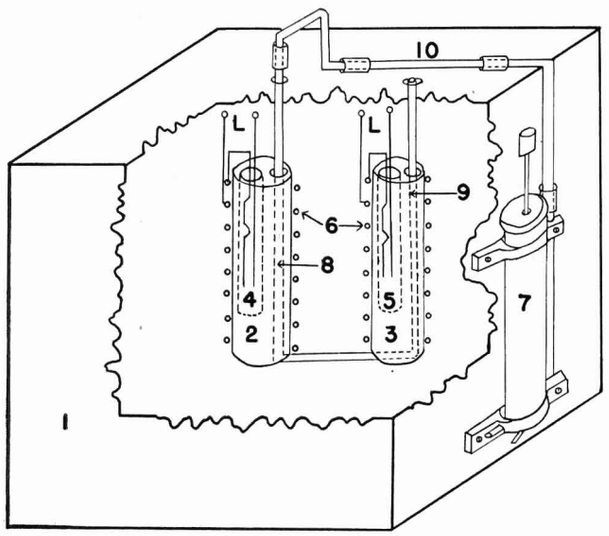

Figure 2. Diagrammatic Sketch of Ethylene Instrument

1. Sheet metal container with glass fiber block insulation 22 cm. long, 21 cm. high, 15 cm. wide
2, 3. Metal block ovens for mercuric oxide and sensitized papers, respectively; 3 cm. in diameter, 10 cm. long
4, 5. Thermoswitches for controlling temperatures of ovens
6. Heating elements; 200 and 50 watts for rapid warm-up, 25 and 8 watts for regulation
7. Gas pipet, 38 cc.
8. Mercuric oxide reaction tube, 285° C., 0.3 cm. inside diameter, 10 cm. long
9. Sensitized paper reaction tube, 125° C., 0.3 cm. inside diameter
10. Absorption trap, if used
L. 115-volt a.c. power supply

The instrument as shown is designed for use on standard 115-volt alternating current power supplies. The mercuric oxide and detecting paper occupy separate arms of a U-shaped glass tube, each arm of which is surrounded by a massive metal block wrapped with two electrical heating elements controlled by a thermoswitch located in a well in the block. This assembly is held in position by providing suitably cut spaces in a rigid form of glass fiber insulation within the metal box container. Pure gum rubber connections are used in connecting the capillary glass tubing to the outlet of the metal gas pipet and to the U-shaped reaction tube. A section of this capillary tubing can be readily removed for inserting traps consisting of short lengths of glass tubing containing suitable absorbents. A suitable glass wool plug at the outlet of the pipet is used to control the rate of flow of the sample from the pipet through the reaction tube. For the dimensions shown in Figure 2, a delivery time of about 2 minutes for the 38-cc. pipet gives an average contact time of sample with mercuric oxide of about 1.5 seconds and a satisfactorily defined boundary between the darkened and unchanged portion of the sensitized paper.

Sensitized Paper. Selenium paper is used in the form of strips 0.125 inch wide and 6 inches long. This paper with calibration properties known within 10% may be prepared as described previously(*26*). Thus, when used with the instrument as described above, sensitized papers prepared from Schleicher and Schuell (American) No. 610 filter paper with 0.010 *M* and 0.10 *M* selenocyanate solutions would show 50-mm. lengths of blackening (after correction for blanks) for samples containing 20 and 200 p.p.m. of ethylene, respectively. For analysis of samples containing over 200 p.p.m. of ethylene, a higher paper temperature than 125° should be used or the sample should first be diluted.

Operation. A strip of sensitized paper is placed in the outlet arm of the U-tube and a standard volume of sample is passed through the reaction tube by means of the gas pipet. This procedure is repeated until three or four successive samples show that a reproducible length of blackening is obtained. A blank value is obtained in the same way, using pure oxygen or air as samples. The length of blackening (corrected for the blank) is proportional to the concentration of ethylene in the sample and is converted to parts per million by use of the calibration constant or calibration curve for the paper used. Lengths of blackening up to 80 mm. may be used.

Performance. The calibration data in Figure 4 of (*26*) were obtained using the instrument and standard samples of ethylene in oxygen. By proper choice of sensitized paper and by separate calibration of each sheet of sensitized paper from which strips are cut, one may make analyses with an error of about 2% or 0.5 p.p.m., whichever is larger. However, errors three to four times as great may result in routine use of the instrument with paper prepared by a standard procedure without calibration of individual sheets. As the blank values obtained correspond to 1 or 2 p.p.m. of ethylene, the instrument is of limited use for estimating ethylene at concentrations below 5 p.p.m.

INTERFERING SUBSTANCES

In addition to ethylene, oxidizable substances that have been found in the volatile constituents of fruits include ethyl alcohol, acetaldehyde, esters of fatty acids, geraniol, and limonene (*4*, *5*, *23*, *24*, *27*). Except for limonene, all these substances are oxygenated derivatives of hydrocarbons and as such are expected to react with concentrated sulfuric acid (*7*, *12*). *d*-Limonene, the principal constituent of orange oil, may also be expected to be reactive with concentrated sulfuric acid because, as a terpene, it is an unsaturated hydrocarbon. The authors therefore investigated the use of sulfuric acid for removing substances typical of those most likely to interfere in field use of the instrument.

As it was known that ethylene is partially absorbed when passed through 95% sulfuric acid (*28*), conditions were sought under which samples containing on the order of 10 to 100 p.p.m. of ethylene could be treated with sulfuric acid without loss of ethylene. As expected from the work of Tropsch and Mattox (*28*), it was found in both gravimetric and paper detection experiments with gas washing bottles of the side inlet fritted disk type that 95 and 90% sulfuric acid remove some ethylene from standard samples containing 100 p.p.m. of ethylene in oxygen, but 86% sulfuric acid does not. However, no ethylene was removed from such a sample when it was passed over pumice saturated with 95% sulfuric acid with average contact time of about 3 seconds. Contact times of the order of 30 seconds were required before a detectable portion (about 5%) of the ethylene is removed by this type of trap.

Granular pumice (10- to 14-mesh) was pretreated by boiling in 95% sulfuric acid, washing in water, and drying in an oven. After saturation in 95% sulfuric acid, the pumice was placed between glass wool end plugs in a 100-mm. length of 7-mm. glass tubing. This trap was inserted between the outlet of the gas pipet and the reaction tube of the instrument.

The effectiveness of these sulfuric acid–pumice traps for removing traces of acetaldehyde, ethyl alcohol, ethyl acetate, acetone, diethyl ether, and acetic acid was studied using the instrument and samples containing on the order of 10 p.p.m. of air. For each substance the trap reduced the length of blackening approximately to that found in blank determinations, indicating that less than one p.p.m. of ethylene equivalent remained in the scrubbed gas stream.

The effect of these 95% sulfuric acid–pumice traps on gaseous samples containing from 20 to 140 p.p.m. of ethylene equivalent of orange oil or *d*-limonene vapor in air was also studied using

the instrument. After passage through a freshly prepared trap these samples contained less than 5 p.p.m. of ethylene equivalent. When such samples were measured on the instrument without passing through a trap, the mercuric oxide reaction tube showed a high residual blank and did not respond quickly to changes in reducing capacity of the sample.

All the above tests were made with about 3 seconds' average contact time of the sample with the sulfuric acid trap. It is recommended that a drying tube be attached to the intake of the gas pipet when the instrument is used with a sulfuric acid trap, to avoid diluting the acid with water vapor in the sample.

In view of the relatively high specificity of the method developed by Young (*29*) for the determination of traces of ethylene by absorption in 0.25 *M* mercuric perchlorate and 2 *M* perchloric acid solution, followed by its release by addition of chloride and measuring the volume of released gas at atmospheric pressure in a Warburg apparatus, absorption in mercuric perchlorate solution and subsequent release by addition of chloride were investigated as a means of making the instrument specific for ethylene. For this purpose a modified 50-ml. syringe was used for successively scrubbing a 40-ml. gas sample with perchlorate solution, followed by addition of sodium chloride solution to release the ethylene back into the scrubbed gas sample. Orange oil vapor-air samples were thus found to have all the oxidizable material removed by the perchlorate solution, but an appreciable proportion of it was subsequently released into the gaseous sample by the addition of chloride. It was concluded that absorption of the ethylene in, and subsequent release from, mercuric perchlorate solution was not a satisfactory method of making the instrument specific for ethylene.

Carbon monoxide is a possible interfering substance which should not be overlooked in practical use of the instrument for estimating ethylene. It is not removed by use of a sulfuric acid trap. When the presence of carbon monoxide is suspected as an impurity, it can be separately estimated by the National Bureau of Standards colorimetric indicating gel method (*25*). One part per million of carbon monoxide present in the sample will give a response with the instrument equivalent to 0.185 p.p.m. of ethylene.

DISCUSSION

Several field tests of the instrument have been made in citrus coloring rooms. In one series of tests no change in length of blackening was found on inserting a fresh 95% sulfuric acid–pumice trap between the pipet and the reaction tube. This indicates that concentrations of the possible interfering substances discussed above were probably not significant relative to the 10 to 30 p.p.m. of ethylene measured in those tests.

In a survey of seventeen citrus packing house coloring rooms, A. H. Rouse and J. C. Bowers of the Florida Citrus Experiment Station, Lake Alfred, Fla., measured ethylene concentrations both by use of the instrument described above and by the bromination micromethod of Hansen (*10*), in each case scrubbing the gas samples first with a 95% sulfuric acid–pumice trap. Omitting one room where the two methods gave results of 105 and 84 p.p.m., the instrument showed on the average 1.3 p.p.m. more ethylene present and a mean absolute difference of 4.2 p.p.m. from the bromination micromethod, the concentrations ranging from about 5 to 100 p.p.m. They also found no consistent difference in measurements made on the instrument with and without the sulfuric acid trap inserted.

A single determination of ethylene by the perchlorate absorption procedure of Young (*29, 30*) was made in a citrus coloring room for comparison with results obtained with the instrument. Approximately 5 hours were required to collect the sample by absorption. This sample showed an ethylene concentration of 8.1 p.p.m., whereas measurement on the instrument during that time gave 12 ± 1 p.p.m. with the sulfuric acid trap inserted.

Further work is required to establish the errors arising from estimating ethylene concentrations in citrus coloring rooms by use of the instrument, but the results already obtained indicate that the instrument should be satisfactory for practical control purposes in this application.

No field tests have yet been made on use of the instrument to estimate ethylene concentrations developing in storage rooms for noncitrus fruits such as apples, pears, or bananas.

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Small Scale Filter Paper Chromatography

Filter Papers and Solvents

LOUIS B. ROCKLAND[1], JEREMIAH L. BLATT, AND MAX S. DUNN
University of California, Los Angeles, Calif.

Investigations were undertaken because of the rapidly growing interest in and importance of filter paper chromatography as a tool for the qualitative identification and quantitative determination of amino acids and other biological substances. Thirteen filter papers have been rated on the basis of seven physical and chemical characteristics, the influence of the paper on the R_f values of eighteen amino acids has been studied, the influence of water content of eight water-miscible solvents on R_f values of amino acids has been investigated, and the R_f values (ratio of the length of the chromatogram to the distance traveled by the solvent boundary) of amino acids representative of the acidic, basic, neutral, and cyclic groups have been determined. The fixed and most common sequences of the amino acids, the solvents yielding inverted sequences, and the solvents most effective in separating individual amino acids, pairs of amino acids, and groups of amino acids have been determined.

SMALL scale filter paper chromatography, described in earlier papers (*16–18*) from the authors' laboratory, has been applied to the study of filter papers (*11*) and solvents (*9*) and has been adapted to the rapid qualitative (*9, 15*) and quantitative (*16*) determination of amino acids. The authors' systematic investigations relating to filter papers and miscible and immiscible solvents are reported in the present paper.

EXPERIMENTAL

The tests were conducted using the capillary ascent test tube method of Rockland and Dunn (*17*).

The solvents were introduced conveniently into the test tubes without wetting the walls by means of a wooden ramp with a V-shaped groove and a 50-ml. buret mounted in such a manner that its 6-inch (15-cm.) sealed-on glass delivery tube was parallel to and nearly touching the groove in the ramp. Test tubes, calibrated by means of a ruled tapered brass strip, were selected which permitted the trapezoidal strip of filter paper to be held in position without touching the walls of the test tube except at the upper end.

A rectangular glass chamber (museum jar No. 4, outside dimensions 15 × 9 × 15 cm.) with three 0.25-inch wooden dowels inserted in the grooves in the jar was found advantageous when it was desired to determine simultaneously the R_f values of a number of amino acids using a single solvent mixture. Analogous types of apparatus for macro chromatography have been described recently by Block (*3*) and Datta *et al.* (*6*). For two-dimensional chromatography it is convenient to have available two museum jars, one for each solvent mixture. The test tube technique is particularly useful for solvent studies and preliminary separation of amino acids, while the museum jar method is more satisfactory for qualitative and quantitative analysis of amino acid mixtures.

The Gilmont ultramicroburet (0.01-ml. capacity) was entirely satisfactory for small-scale chromatography of amino acids which requires that spots of minimum size be introduced on the filter paper. Uniform and relatively high precision in the delivery of solutions was accomplished by means of an aluminum ($1/16$-inch) platform (2.5 × 7.5 inches) attached to an auxiliary rod supporting the buret and provided with a lever with which it could be raised to make contact with the tip of the buret at any position of the filter paper. Using these devices ten aliquots of solution could be added to the paper without increasing the initial spot size. A single spot (approximately 10^{-4} ml. of solution) dries completely within 1 minute when exposed to a gentle stream of warm air.

The initial position of an amino acid spot was marked accurately with a pencil held in a 3-inch piece of aluminum tubing 0.375 inch in inside diameter, fixed to the buret tip with Scotch tape.

[1] Present address, Fruit and Vegetable Chemistry Laboratory, U. S. Department of Agriculture, Pasadena, Calif

The exact final positions of the solvent boundaries were located in some instances under ultraviolet light. Chromatograms developed with volatile solvents were dried in air, and those developed with phenol and other higher boiling solvents by heating them for 5 minutes in an oven at 80°. It was found convenient to store paper strips spotted with standard solutions of amino acids in stoppered 25 × 200 mm. test tubes and paper sheets spotted similarly in letter file folders.

In staining chromatograms solvents were employed in which the chromogenic reagent, but not the amino acid, was appreciably soluble. The solutions found most useful were 0.5% ninhydrin in dry methyl Cellosolve and 4% ninhydrin in pyridine

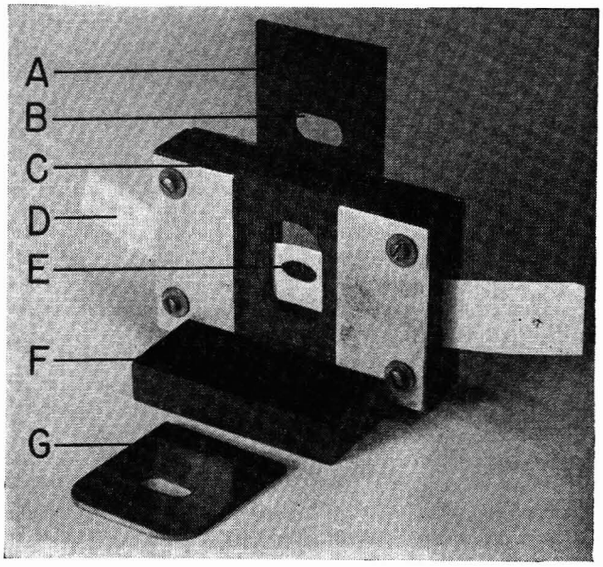

Figure 1. Sample Holder for Photometric Determination of Ninhydrin-Stained Amino Acid Chromatograms in Small-Scale Chromatography

A. Rear Bakelite columnating piece (raised out of position)
B. Elliptical columnating opening, $1/4 \times 1/2$ inch. Other columnating pieces with elliptical opening $1/4 \times 5/8$, $3/8 \times 5/8$, or $3/16$ inch × $3/8$ inch, and with circular opening $7/16$, $3/8$, $5/16$, or $1/4$ inch in diameter have been found useful
C. Sample holder constructed of two Bakelite strips, each $1/4 \times 2\,3/4 \times 1\,5/8$ inches, joined and held in position with four aluminum strips, each $1/16 \times 1\,5/8 \times 7/8$ inch. Sample holder set in a Bakelite block $2 \times 3/8 \times 1\,3/4$ inches
D. Filter paper strip
E. Ninhydrin stained spot
F. Sample holder base
G. Front Bakelite columnating piece (when in position occupies milled slot in front of spot on filter paper strip)

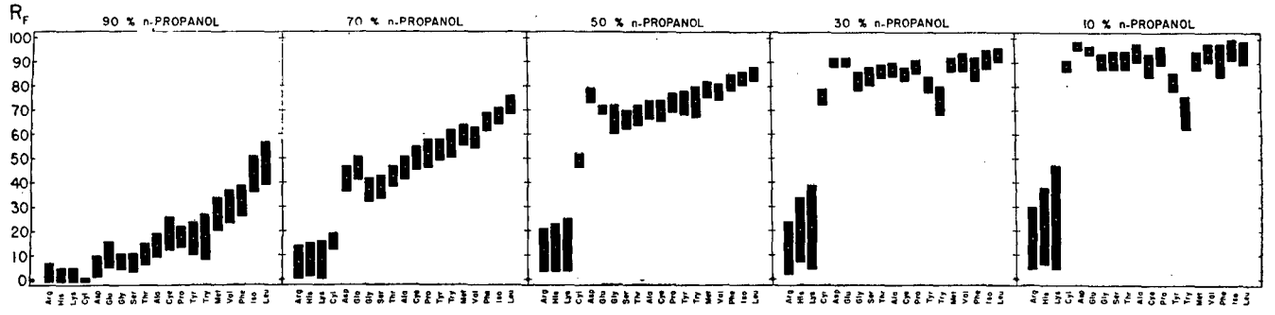

Figure 2. R_f Values of Amino Acids in 1-Propanol–Water Mixtures

Arg (arginine), His (histidine), Lys (lysine), Cyi (cystine), Asp (aspartic acid), Glu (glutamic acid), Gly (glycine), Ser (serine), Thr (threonine), Ala (alanine), Cye (cysteine), Pro (proline), Tyr (tyrosine), Try (tryptophan), Met (methionine), Val (valine), Phe (phenylalanine), Iso (isoleucine), and Leu (leucine)

(Eastman Kodak Co., white label). Rockland and Miller (*19*) have shown that use of the latter solvent facilitates the discernment of faint spots and, because the spots develop gradually at room temperature, the differentiation of amino acids present in relatively high concentration or in close proximity in chromatograms.

Uniform staining of the chromatograms was accomplished by immersing each paper strip in about 35 ml. of staining solution contained in a 20 × 150 mm. test tube and by drawing each paper sheet or series of paper strips through the staining solution in a glass baking dish. This technique conserves ninhydrin, as well as time, and permits more uniform application than spraying.

The R_f values of the chromatograms were estimated with the aid of the partogrid (*18*). The transmittance characteristics (Table I) of the filter papers were determined quantitatively using a sample holder (Figure 1) (constructed to hold a filter paper strip in the milled groove between Bakelite blocks) which was inserted in the sample compartment of a photoelectric colorimeter (Lumetron 402-E) (*16*).

Table I. Filter Paper Characteristics

Type[a]	No.	Texture[b]	Solvent Boundary[c]	Uniformity[d]	Weight, Mg./sq. cm.	Solvent Speed[e], Min.	Ninhydrin Color[f]	Resolving Power[g], R_p
S. & S. 589 blue	1	B	A	A	11	260	Blue gray	9
S. & S. 507	2	A	A	A	11	240	Blue gray	10
S. & S. 589 red	3	B	A	A	11	180	Blue gray	11
S. & S. 602 E. & D.	4	B	A	A	11	270	Purple	12
Whatman 1	5	B	A	B	11	190	Purple	12
S. & S. 602	6	B	A	A	11	280	Pink purple	13
S. & S. 576	7	A	A	A	11	280	Blue gray	14
Munktells O	8	C	A	B	8	60	Pink rose	14
S. & S. 598 YD	9	B	A	A	13	100	Pink	15
E. & D. 7	10	B	B	C	9	120	Pink	16
Munktells IF	11	D	B	B	9	80	Pale pink	15
E. & D. 248	12	D	C	B	11	240	Purple	13
E. & D. 613	13	D	C	A	9	180	Pink	15

[a] S. & S., Schleicher and Schuell; E. & D., Eaton and Dikeman.
[b] A, smooth; B, medium rough; C, rough; D, very rough.
[c] A, even; B, uneven; C, very uneven.
[d] Based on per cent transmittances, each the average of four values determined with a photoelectric colorimeter (Lumetron 402 EF) and the sample holder (shown in Figure 1) over four different areas of a trapezoidal strip of filter paper. Values found were S. & S. 598 YD 43, E. & D. 248 46, S. & S. 589 blue 47, E. & D. 613 58, E. & D. 7 60, and all others 49–54. Mean deviation from mean values, A, less than 1%; B, 1 to 2%; C, more than 2%.
[e] Time required at 26° for water-saturated phenol to ascend 120 mm. on trapezoidal filter paper strips. Each value is average of 5 to 10 closely agreeing replicate determinations.
[f] Most common color observed for chromatograms of individual amino acids stained by spraying paper strips with 0.25% ninhydrin in water-saturated butanol and heating sprayed strips for 5 minutes at 80°.
[g] Ratio of length of stained amino acid chromatogram to distance traveled by solvent boundary. Each value is average of values found for 12 different amino acids each spotted with 10^{-4} ml. of 0.03 *M* solution on trapezoidal filter paper strip.

Table II. R_f Values of Amino Acids on Different Filter Papers

Amino Acid	Filter Paper Number[a] 1	2	3	4	5	6	7	8	9	10	11	12	13	Range
Aspartic acid	41	21	31	22	25	22	23	28	36	30	24	20	20	20–41
Glutamic acid	53	38	48	39	40	38	38	42	46	46	37	28	32	28–53
Lysine	24	24	30	46	42	50	25	65	50	50	64	71	60	24–71
Arginine	42	31	45	58	58	52	34	78	64	61	68	73	62	31–78
Histidine	44	50	50	56	53	56	45	73	62	57	65	77	76	44–77
Cystine	35	31	35	36	36	34	30	48	45	43	45	38	38	30–48
Serine	44	41	48	44	42	44	38	53	52	48	54	48	49	38–54
Glycine	47	42	49	49	48	44	43	60	58	57	60	53	52	43–60
Threonine	56	54	60	56	58	56	50	65	66	64	70	62	62	50–70
Cysteine	67	61	64	65	64	62	58	73	73	72	73	69	67	58–73
Alanine	66	62	66	66	66	65	60	75	75	71	76	71	68	60–76
Tyrosine	67	61	64	67	68	66	63	77	76	73	79	65	69	61–79
Valine	80	79	82	83	82	82	82	92	86	87	90	78	87	78–92
Methionine	83	80	84	84	83	83	84	92	86	84	94	88	87	80–94
Leucine	86	86	88	90	91	88	83	94	91	..	89	82	89	82–94
Isoleucine	88	86	87	86	88	87	85	94	91	93	94	86	89	85–94
Phenylalanine	87	85	88	88	90	87	83	94	91	92	95	90	95	83–95
Proline	91	95	72	91	88	91	87	93	97	92	95	77	92	72–95

[a] See Table I for filter paper types. Solvent was water-saturated phenol.

DISCUSSION

Filter Paper Studies. Thirteen types of filter papers are listed in Table I approximately in the order of their suitability for use in amino acid chromatography as determined on the basis of seven characteristics. These results are in good agreement with the less comprehensive investigations of Kowkabany and Cassidy (*11*) and Bull *et al.* (*4*). The latter authors have pointed out that slow solvent speed is one criterion of the most satisfactory papers.

The R_f values for 18 amino acids determined on 13 filter papers using water-saturated phenol at 26° as solvent are shown in Table II. It is of interest that with only a few exceptions the order of arrangement of the monoaminomonocarboxylic acids is independent of the type of filter paper; the R_f values obtained with the different papers were somewhat variable for the dicarboxylic and the basic amino acids; the sequence of arginine and histidine is reversed on different papers; and the value for aspartic acid differed widely from those for the other amino acids on each of the filter papers except numbers 1, 2, 3, and 7. Other amino acids for which separations on certain papers (numbers given in parentheses) appear to be possible include glutamic acid (8, 11, 12, 13), lysine (1), histidine (5), cystine (1, 7, 12, 13), serine (11, 12), glycine (5), threonine (12), and proline (2, 3, 9).

The shades of the ninhydrin colors for individual amino acids varied on different papers and the R_f values for lysine increased as the colors changed from blue gray to light pink. As it was observed (data not shown) that the pH values of aqueous extracts of the filter papers varied from

Table III. Amino Acid Sequences Based on R_f Values in Different Solvents[a]

Group	Sequence: Fixed	Sequence: Most common	Solvent Yielding Inverted Sequence	Solvent Most Effective in Separating Amino Acid Pair
Acidic	Glutamic acid > aspartic acid			6, 11
Basic		Histidine > arginine	7, 8, 11	5, 7, 8, 9, 11
		Histidine > lysine	7, 8, 11	5, 6, 9, 10, 11
		Arginine > lysine	5	6, 8
Neutral	Serine and glycine > cystine			3, 4, 5, 7
		Serine > glycine	1, 6, 7, 8, 9	7, 8, 11
	Threonine > serine and glycine			4, 5, 6
	Alanine > threonine			7, 8, 9
	Valine and methionine > alanine			3, 4, 6, 7, 8, 9, 10
		Methionine > valine	5, 11	2, 8, 9, 11
	Isoleucine and leucine > methionine			3, 8, 11
	Leucine > isoleucine			2, 8, 9, 11
Cyclic		Proline > tyrosine	1, 2, 10, 11	6, 7, 8, 9, 10
		Phenylalanine > tyrosine	10, 11	1, 3, 6, 7, 8, 9, 4
		Tryptophan > tyrosine	5	6, 8, 9
		Phenylalanine > tryptophan	10, 11	3, 5, 8
		Phenylalanine > proline	5, 6	1, 2, 3, 4, 10, 11

[a] See footnotes to Figure 6 for notations. Underlined figures indicate solvents which yield greater (or greatest) differences in R_f values.

4.8 to 6.5 and that there were marked differences in the ninhydrin colors of chromatograms on the filter papers, these factors may account for the variability of the R_f values of the acidic and the basic amino acids. In general, the more acidic pH values were correlated with the deeper ninhydrin colors. The significance of pH was further denoted by tests of lysine on S. & S. 589 Blue Ribbon paper with phenol as solvent. The R_f values were 0.25 for untreated paper, 0.37 and 0.50 for paper treated with 0.005 N sodium hydroxide, and 0.42 for paper treated with 0.005 N hydrochloric acid. McFarren (*12*) has shown recently that all the naturally occurring amino acids may be separated on a one-dimensional chromatogram by means of water-immiscible solvents and filter papers buffered to particular pH values.

Solvent Studies. In tests on solvents containing 1-propanol or other types of water-miscible alcohols and hydrochloric acid utilized by Moore and Stein (*13*) for the separation of amino acids on starch columns, the liquid phases formed two widely separated boundaries on filter paper strips and the amino acids did not migrate beyond the lower solvent boundary. On the other hand, there was only one solvent boundary and the amino acids separated satisfactorily when hydrochloric acid was omitted from these solvents. Success in resolving amino acids under these conditions depends, it would appear from the experiments of Consden *et al.* (*5*) on water-miscible solvents, on the relatively low water content of papers not subjected to preliminary equilibration with aqueous solvents. Water logging of filter papers encountered in the use of water-saturated volatile organic liquids was prevented by Bentley and Whitehead (*2*) and other workers (*1*, *7*, *8*, *10*) through the use of air-tight chambers or reduced air space.

The R_f values of 19 amino acids with five solvent mixtures containing from 10 to 90% 1-propanol in water are shown in Figure 2. Cystine separated completely from all other amino acids with 50% 1-propanol and tryptophan with 10% 1-propanol. It should be possible, therefore, to separate cystine and tryptophan quantitatively under these conditions and to determine them quantitatively by the direct photometric method of Rockland and Dunn (*16*) or by some analogous procedure.

The R_f values of asparagine (tan colored spot with relatively small R_f value) and of leucine (purple spot with relatively high R_f value) with solvent mixtures containing from 10 to 90% water in various alcohols (methanol, ethanol, 1-propanol, isopropyl alcohol, and *tert*-butyl alcohol) and Cellosolves (methyl,

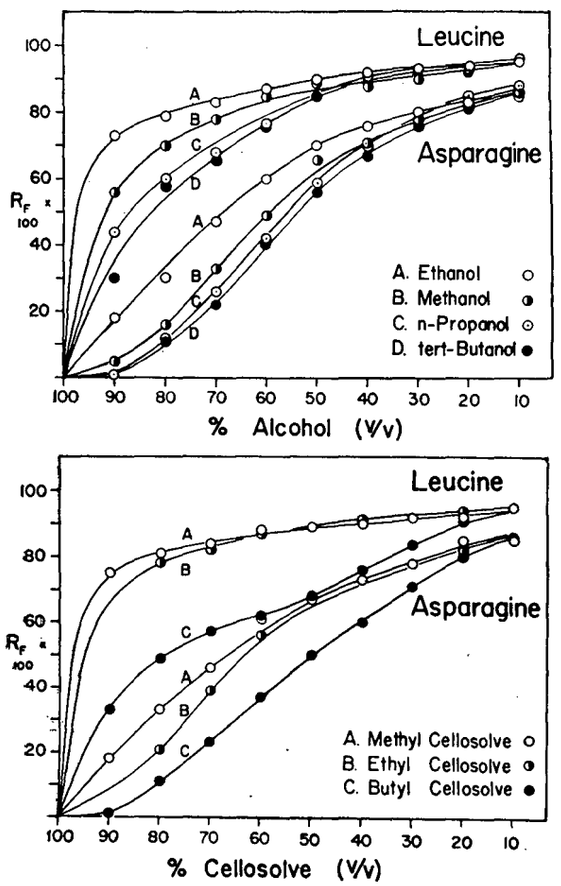

Figure 3. R_f Values of Leucine and Asparagine in Aqueous Alcohols and Aqueous Cellosolves

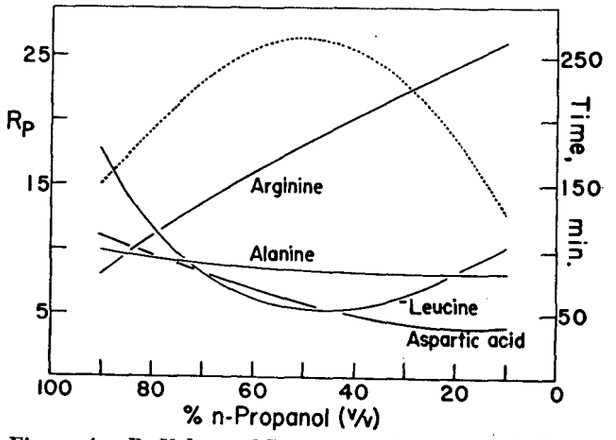

Figure 4. R_p Values of Representative Amino Acids in 1-Propanol–Water Mixtures

.....Running time (minutes) of solvents on S. & S. 589 Blue Ribbon filter paper

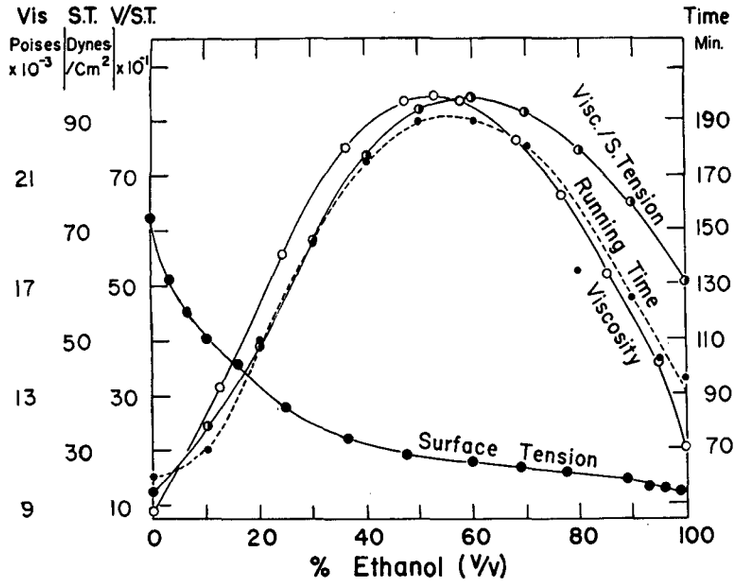

Figure 5. Correlation of Running Time of Aqueous Ethanol Solutions on Filter Paper with Viscosity and Surface Tension of Solvents

ethyl, and *n*-butyl Cellosolves) are shown in Figure 3. The values increased regularly with increasing percentage of water in the solvent mixture and decreased with increasing number of carbon atoms in the molecule of organic substance. The greatest spread in the R_f values occurred over the 10 to 30% range of water concentration. Methanol was found to give the greatest spread in R_f values and butyl Cellosolve the least. Combinations of alcohols or Cellosolves in water yielded R_f values intermediate between those for the individual solvent-water pairs.

Table IV. Solvents Most Effective in Separating Groups of Amino Acids and Individual Amino Acids in a Group

	Group			
Group	Acidic	Basic	Cyclic	Neutral
Neutral	7	5, 11[a]	6, 7, 8	6, 7, 8, 9
Cyclic	4, 6, 7, 8, 9	3, 4, 5, 9, 10, 11	4, 8, 9	
Basic	5, 6, 7, 8	6, 7, 8, 10		
Acidic	6, 11			

[a] Underlined figures indicate solvents which yield greater (or greatest) differences in R_f values. See footnotes to Figure 6 for notations.

The R_p values (see footnote g, Table I) of the amino acids varied with the percentage of water in the solvent mixture. As shown in Figure 4 for four groups of amino acids (only one representative of each group listed), the R_p values of arginine (also histidine and lysine) decreased linearly with increasing propanol in the solvent mixture, the R_p values of aspartic acid (also glutamic acid) increased linearly in the solvent mixtures containing more than 50% 1-propanol, the R_p values of leucine (also cysteine, isoleucine, valine, phenylalanine, and tryptophan) were minimal at about 50% propanol, and the R_p values of alanine (also glycine, serine, and threonine) did not vary significantly with changing solvent composition. The lowest values for leucine were found with the alcohols and Cellosolves of highest molecular weights and for the solvent mixtures of the percentage composition which gave the longest running times. It was also determined (Figure 5) that the solvent running time for solvent mixtures of different ethanol concentrations nearly paralleled the ratios of the viscosity and surface tension of these solvent mixtures.

[Since this work was completed and the manuscript submitted for publication, Müller and Clegg (*14*) have reported that the density, as well as the viscosity, of the solvent is a significant factor in retarding solvent travel. If the curve (Figure 5) for viscosity–surface tension is modified to $\frac{\text{viscosity} \times \text{density}}{\text{surface tension}}$, it more closely approximates the shape of the solvent velocity curve in agreement with the findings of Müller and Clegg.]

It is of practical importance that there was no significant change in the R_f values of alanine or glycine determined from four chromatograms of each amino acid prepared successively with the same aliquot of water-saturated phenol on trapezoidal strips of Whatman No. 1 paper. The R_f values were also identical when determined similarly with a fresh aliquot of this solvent in the unwashed test tubes. The R_f values for each of 19 amino acids were identical when determined on trapezoidal strips in test tubes and a museum jar, but differed significantly from those obtained on rectangular sheets of paper in a museum jar. In general, the R_f values were higher and the running times longer for the trapezoidal strips than for the rectangular sheets of filter paper, especially for the acidic and the basic amino acids.

The fixed and most common sequences of amino acids, the solvents yielding inverted sequences, and the solvents most effective in separating individual amino acids, pairs of amino acids, and groups of amino acids are shown in Tables III and IV. As shown in Figure 6, curves drawn from plots of the R_f values in eleven solvent mixtures for amino acids representative of the acidic, basic, neutral, and cyclic groups are strikingly similar in

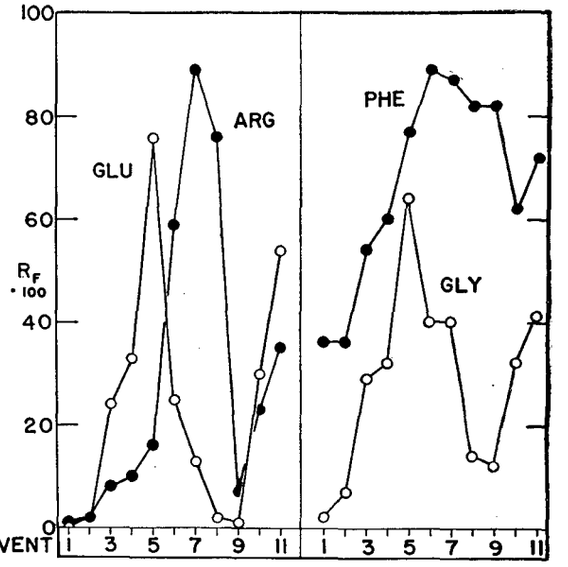

Figure 6. R_f Values of Representative Acidic, Basic, Neutral, and Cyclic Amino Acids in Different Solvents

Whatman No. 1 filter paper
Solvents and methods.
1. Water-saturated benzyl alcohol. Descending, large scale (5)
2. *tert*-Amyl alcohol (5)
3. *tert*-Butyl alcohol, 70%. Ascending, small scale sheets, museum jar technique
4. 1-Propanol, 70% as in 3
5. Methanol, 67% as in 3
6. Water-saturated phenol with HCN (5)
7. Water-saturated phenol with 3% ammonia (5)
8. Water-saturated *m*-cresol with cupron and 0.1% ammonia (5)
9. Water-saturated *m*-cresol with cupron (5)
10. Collidine-lutidine. Ascending, museum jar
11. Tetrahydrofurfuryl alcohol, 75%

shape but with displaced maxima and minima. That there was a fixed order of the R_f values found in different solvent mixtures for some, but not all, of the amino acids of a group was determined by inspection of their superimposed curves drawn on transparent plastic sheets. The importance of pH is emphasized by the relatively large differences in the R_f values of the acidic and the basic amino acids with the solvent pairs No. 5 (methanol-water) and No. 6 (phenol-water with hydrocyanic acid), No. 6 and No. 7 (phenol-water with ammonia), and No. 8 (*m*-cresol-water with ammonia) and No. 9 (*m*-cresol-water).

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Quantitative Determination of Sugars on Filter Paper Chromatograms by Direct Photometry

EARL F. McFARREN, KATHLEEN BRAND, AND HENRY R. RUTKOWSKI
National Dairy Research Laboratories, Inc., Oakdale, L. I., N. Y.

Neither conventional methods of sugar analysis nor paper chromatographic elution methods permit the quantitative determination of galactose and glucose in the presence of one another with any reasonable assurance of accuracy. Sufficient separation of galactose and glucose can be achieved on a paper chromatogram to permit the quantitative determination of each sugar by direct photometry. In this method the maximum densities of the developed sugar spots are determined by means of a densitometer and a standard curve is prepared by plotting the logarithm of the concentration against the densities. The densities of the unknown sugar spots are similarly determined and their concentrations calculated from the standard curve. Data are presented which indicate that it is possible to determine the sugars present in a mixture with an error no greater than 5%. Quantitative analysis of samples by chromatography has revealed the presence of reducing substances not suspected of being present, which apparently have been calculated as lactose or total monoses in the usual sugar analysis.

THE method presented here for quantitatively determining the sugars is essentially the same as the methods published by Block (*2*) and Rockland and Dunn (*10*) for quantitatively determining the amino acids by direct photometry on filter paper chromatograms. The method of Rockland and Dunn requires determination of the density of the entire spot, while Block's method, as in the present study, requires determination of only the maximum density of the spot. This study was begun before either of these methods was published and was developed as a result of a suggestion during a discussion with R. J. Block.

Both Block (*3*) and Bull *et al.* (*5*) published separately at about the same time another method for quantitatively determining the amino acids. In this method the density of consecutive 5-mm. segments of a strip chromatogram was determined using an electron transmission densitometer. The densities determined along the strip were plotted against the distance from the starting point and curves were drawn. Block pointed out that when such a curve was plotted it could be shown that the peaks of the curves varied in height with the concentration, the indication then being that there might be a simple relationship between the maximum density and the concentration.

Fisher, Parsons, and Morrison (*6*) have shown experimentally that a linear relation holds between the area of the spot of test substance and the logarithm of the concentration at which it is originally applied.

From Beer and Lambert's law it is known that in a solution the concentration is proportional to the density. At first thought this relationship would seem to apply here. However, Brimley (*4*) in developing a theoretical derivation of the relationship of the area of a spot to the concentration supposed that the spot spread by diffusion as it moved along the chromatogram. Making this assumption, by analogy, it would seem to follow that the density of a spot on the chromatogram is linearly related to the log of the concentration.

Block (*1*) has since shown that this relationship holds experimentally for the amino acids. This relationship is shown here also to hold experimentally in the case of sugars.

Briefly, the method employed consists of separating the sugars in an ethyl acetate–pyridine-water solvent system containing silver nitrate, air drying, exposing the chromatograms to ammonia vapors, and developing the sugar spots by heating in an oven. The maximum densities of the developed spots are then determined by means of a densitometer, and a standard curve is prepared by plotting the log of the concentrations against the densities. The densities of the unknown sugar spots are determined and their concentrations are calculated from the standard curve.

Table I. Expected Error in Calculation of Concentration of Sugars from Density Readings Determined by Direct Photometry on Filter Paper

Sugar	Standard Concn., Mg./2 Ml. 5	7.5	10	12.5	Unknown Dilution 1/4	1/3	1/2	Calculated Concn., γ/μ.	Average, G./100 Ml.	Theoretical, G./100 Ml	% Error
Lactose	0.53	0.91	1.17	1.31	0.61	0.81	1.16	10.25	1.02	1.00	2.0
	0.52	0.92	1.17	1.21	0.58	0.87	1.13	9.83	0.98	1.00	2.0
Galactose	1.12	1.58	1.74	1.84	1.01	1.22	1.52	7.95	0.79	0.80	1.0
	0.92	1.41	1.64	..	0.81	1.11	1.39	8.02	0.80	0.80	2.0
Glucose	1.49	1.97	2.00	2.20	1.19	1.46	1.93	7.42	0.74	0.76	3.0
	1.27	1.76	1.95	..	1.13	1.41	1.69	7.17	0.72	0.76	5.0

EXPERIMENTAL PROCEDURE

The solvent system employed was prepared by placing 2.5 parts of ethyl acetate, 1.0 part of pyridine, and 3.5 parts of distilled water in a separating funnel and shaking thoroughly. After separation, the water-rich layer was discarded and a portion of the solvent-rich layer was placed in the bottom of a cylindrical borosilicate glass chamber 24 inches (60 cm.) high and 12 inches (30 cm.) in diameter. The chamber was lined with two sheets of Whatman No. 1 filter paper stapled together to form a cylinder which fits snugly against the chamber wall. The remainder of the solvent-rich layer was made 0.15 *N* with respect to silver nitrate and placed in a stainless steel solvent trough which was held 22 inches from the bottom of the chamber by a stainless steel stand. The chambers were covered with a 12-inch square of plate glass ground on one side.

The filter paper used was Schleicher and Schuell 589 White Ribbon cut in 22 by 55 cm. rectangular strips. The paper was cut so that the long axis ran parallel with the watermarks. Four standard solutions containing, respectively, 5, 7.5, 10, and 12.5 micrograms per microliter each of lactose monohydrate, glucose, and galactose, were prepared. These sugar solutions were applied in 2-microliter quantities by means of a Gilmont ultramicroburet. Seven spots were introduced along a line 7.5 cm. from one end at 2.5-cm. intervals. The first four spots were the known sugar concentrations used to establish the standard curve, and the other three spots were the unknown solutions applied at appropriate dilutions.

After spotting the paper, the chromatograms were placed in the chambers and allowed to run for 16 to 20 hours. In this period of time, the solvent front had progressed beyond the end of the paper and a better separation of the sugars resulted. Upon removal from the solvent chamber, the chromatograms were air-dried for 1 hour and then placed in an ammonia chamber for 1 hour. The chambers used for the ammonia were the same kind as those used for the chromatographic run. Concentrated ammonia was placed in the bottom of a lined chamber, and the chromatograms were hung on the glass rod of the chromatographic stand (without the trough) by means of a stainless steel clip. On removal from the ammonia chamber, the chromatograms were placed in a mechanical convection oven at a temperature of 80° ± 1° C. for 20 minutes.

The densities of the developed chromatogram (Figure 1) were then determined with a standard Photovolt electron transmission densitometer, using a 5-mm. diameter aperture and the No. 440 filter purchased with the instrument. This filter gives maximum absorption and the highest density readings (Figure 2).

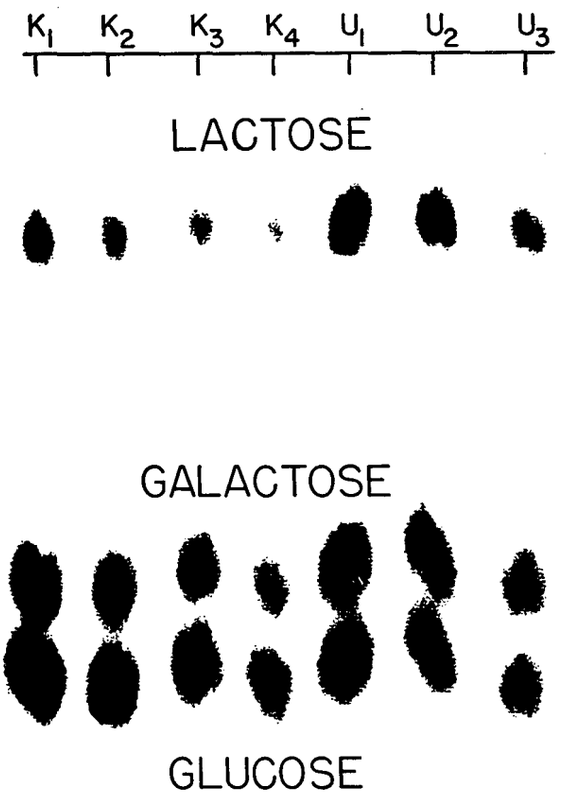

Figure 1. Photocopy of Quantitative Sugar Chromatogram

***K*'s. Knowns applied at concentrations of 15/2, 10/2, 7.5/2, and 5γ/2 μl., respectively, left to right**
***U*'s. Unknowns, in this example spotted repeatedly in 2-μl. portions. A total of 20, 15, and 10 μl. were applied to U_1, U_2, and U_3, respectively**

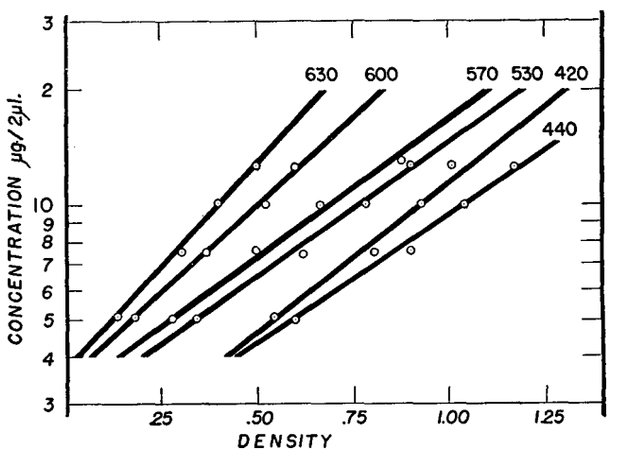

Figure 2. Variation of Density with Filter Used in Densitometer

This instrument was modified slightly by removing the opal glass from the receiving cone and by disconnecting the spring which normally holds the search head arm in a raised position. A cork was placed under the search head arm at the middle of its length and high enough to leave a space between the receiving cone and the aperture disk of about the thickness of two sheets of paper. The shutter spring was also removed, so that it was not necessary to hold the shutter open while trying to determine the density of the spots. The instrument was first zeroed on a blank portion of the developed chromatogram, and then the maximum density of each sugar spot was determined by moving each spot slowly around underneath the receiving cone until maximum deflection was obtained on the density meter. The maximum density is easily found and can readily be reproduced for any one spot, if too long a time has not elapsed between readings. On exposure to light and volatile reducing substances in the atmosphere, over-all darkening of the chromatogram occurs with the passage of time. After the densities have been determined, the data are plotted on semilogarithmic paper with concentration on the log scale as ordinate and density as abscissa. A standard curve is typified by the data plotted for the 440 filter in Figure 2. From this standard curve and the recorded densities of the unknown, the concentrations of the unknown are then calculated.

For evaluating this procedure, a mixture containing 1% lactose, 0.80% galactose, and 0.76% glucose was prepared and run

as an unknown. In this case it was found by trial that it was necessary to run the unknown at dilutions of one-half, one-third, and one-fourth. The data obtained and the calculations, including the per cent error, are recorded in Table I.

DISCUSSION

A number of factors influence the success of this method and cannot be ignored if satisfactory chromatograms are to result.

Solvent. The solvent system employed was that used by Jermyn and Isherwood (*7*). However, the solvent proportions suggested did not always produce good separation of galactose and glucose, and irreproducible R_f values were obtained. It was absolutely essential to have airtight chambers, as even the smallest break in the seal between the chamber and lid would cause variations in R_f values. R_f values varied with the size and number of strips or sheets placed in the chromatogram chamber at one time. In order to obtain good separation of galactose and glucose, it was found necessary to change the solvent proportions of ethyl acetate, pyridine, and water to 2.5:1:3.5. Reproducible R_f values were obtained by lining the chamber with two sheets of Whatman No. 1 filter paper stapled together to form a cylinder and by using the solvent-rich layer (same as in the trough) in the bottom of the chamber. When this was done, reproducible R_f values resulted, regardless of the size of the sheet.

Table II. Variations of R_f Values of Sugars in Ethyl Acetate–Pyridine–Water with Vapor Content of Chamber and Size of Chromatogram

	Chromatogram Size, 11 × 55 Cm.					
	Phase in Bottom, Lined Chamber			Phase in Bottom, Unlined Chamber		
	Solvent rich	Water rich	Water	Solvent rich	Water rich	Nothing
Lactose	0.12 ± 0.02	0.40 ± 0.20	0.18 ± 0.03	0.18 ± 0.03	0.24 ± 0.12	0.30 ± 0.20
Galactose	0.22 ± 0.03	0.47 ± 0.18	0.32 ± 0.04	0.32 ± 0.04	0.36 ± 0.17	0.45 ± 0.13
Glucose	0.27 ± 0.04	0.51 ± 0.17	0.37 ± 0.04	0.37 ± 0.04	0.41 ± 0.16	0.48 ± 0.16
Ribose	0.43 ± 0.03	0.62 ± 0.10	0.55 ± 0.04	0.55 ± 0.04	0.56 ± 0.11	0.55 ± 0.17
	Chromatogram Size, 22 × 55 Cm.					
	Phase in Bottom, Lined Chamber			Phase in Bottom, Unlined Chamber		
	Solvent rich	Water rich	Water	Solvent rich	Water rich	Nothing
Lactose	0.10 ± 0.01	0.20 ± 0.03	0.34	0.12 ± 0.03	0.10 ± 0.00	0.20 ± 0.02
Galactose	0.20 ± 0.01	0.20 ± 0.01	0.40	0.25 ± 0.06	0.21 ± 0.01	0.34 ± 0.01
Glucose	0.24 ± 0.00	0.24 ± 0.00	0.52	0.29 ± 0.05	0.25 ± 0.03	0.38 ± 0.01
Ribose	0.42 ± 0.01	0.42 ± 0.01	0.70	0.49 ± 0.06	0.46 ± 0.02	0.59 ± 0.02

The R_f values obtained in chambers prepared in a number of ways and on sheets of different sizes are recorded in Table II. Reproducible R_f values were obtained only when the solvent-rich layer was used in the bottom of a lined chamber. Chambers prepared in this manner could be used day after day with only the addition of more solvent to the trough prior to the introduction of the filter paper strips; it was not necessary to equilibrate chromatograms overnight. The data in Table II indicate the necessity of assuring saturation of the chambers with both water and solvent. Lining the chambers appears to help in this respect, but because of the volatility of this particular solvent, it is difficult to keep the chamber saturated with solvent vapor unless the solvent-rich layer is also used in the bottom.

Development of Spots. It has not proved feasible for quantitative purposes to spray the chromatograms with ammoniacal silver nitrate, for the water in the spray caused diffusion and spreading of the spots in an unpredictable manner. This difficulty was eliminated by incorporating the silver nitrate in the chromatograming solvent, as had been done by Nicholson (*9*) with ninhydrin for chromatograming the amino acids. After the chromatograms have been removed from the chambers and thoroughly air-dried, it is necessary to expose the sugar chromatograms to ammonia vapor. This has been accomplished by hanging the strips in a lined chamber containing concentrated ammonia in the bottom of the chamber. A 1-hour exposure is required to obtain maximum development of the spots. It is necessary to add 10 to 20 ml. of concentrated ammonia to the ammonia chambers every day when the ammonia chambers are in continual use. If this is not done, the concentration of the ammonia vapor is so reduced by opening and closing the chambers to add or remove chromatograms that the intensity of the developed spots will diminish. Immediately on removal from the ammonia chambers, the chromatograms are placed in a mechanical convection oven at 80° C. for 20 minutes. This temperature and time are rather critical, as a higher temperature will cause greater background coloration and a lower temperature will not develop the sugar spots.

Background Coloration. In an effort to evaluate the suitability of various filter papers, the R_f values for the sugars were determined, using a number of grades and makes of filter paper. Little significant variation in the R_f values was noted regardless of the filter paper used. Schleicher and Schuell 589 White Ribbon was finally chosen, for it gives a desirable rate of solvent flow (about the same as Whatman No. 1) and it is an ash-free paper which gives less background color with ammoniacal silver nitrate. Reducing substances (such as formic acid) in the atmosphere during the air drying of chromatograms will also cause greater background color to develop and subsequently interfere in determining the densities for quantitative purposes. In running a great number of chromatograms, it has been found desirable (after air drying for 1 hour) to hang the chromatograms in an empty air-tight chromatogram chamber until they can be developed. This will prevent or considerably reduce darkening. It is better, however, to stagger the chromatographic runs, so that only one chromatogram is removed from the solvent chambers every 30 minutes. This procedure allows time for the chromatograms to be processed one at a time without delay and the resulting darkening.

Density Values. In general, density readings between 0.50 and 1.70 have given the best results—namely, a linear relationship. Under the conditions specified for developing the chromatograms, glucose has greater reducing properties than galactose which in turn are greater than lactose. As a result, similar density readings will not be obtained for equivalent concentrations of each sugar. The standard curves are not reproducible from one paper strip to another, and the standards must be run each time on the same chromatogram with the unknowns. If the densities of the unknown are not within the same range as the densities of the known, they should be discarded and the chromatograms run again after concentrating or diluting the unknown as indicated by the initial run. Concentration can be done on the paper by repeated applications of the same volume on the same spot after air drying or drying under an infrared lamp after each application.

APPLICATION TO MATERIALS OF BIOLOGICAL ORIGIN

In working with materials of biological origin, it is necessary to remove interfering substances such as proteins and salts. The removal of proteins may be effected in a number of ways. Of the many methods tested, the most effective method was the precipitation of the protein with barium hydroxide and zinc sulfate as used by Somogyi (*11*) for blood clarification. In samples deproteinized the amount of reagents added varied with each sam-

ple; however, it was necessary to add for each equivalent of zinc sulfate an equivalent of barium hydroxide.

The barium hydroxide and zinc sulfate solutions were made approximately 0.3 *N* and the equivalents were determined by titrating the zinc sulfate solution with the barium hydroxide to a phenolphthalein end point. A sample of spray-dried skim milk was deproteinized by adding 20 ml. of zinc sulfate and 17.2 ml. of barium hydroxide (1.00 ml. of zinc sulfate is equivalent to 0.86 ml. of barium hydroxide) to 1 gram of sample dissolved in 50 ml. of water. The sample was then diluted to 100 ml. by adding distilled water. After gently shaking, the solution was filtered through Whatman No. 12 fluted paper and a portion of the filtrate was used for chromatograming.

Each sample under investigation is an individual case, and the amount of reagent used, and the extent of dilution, vary with the amount of sample to be deproteinized. To obtain a rapid filtration and a clear filtrate which marks an effective deproteinization, a pH of 7.2 to 7.6 is required in the sample-reagent mixture prior to filtration. This procedure has been applied to whole, dried, and skim milks, to egg whites, to cheese, and to tissue and body fluid extracts of animals. Satisfactory undistorted chromatograms suitable for quantitative determinations have resulted in nearly all cases. Some interference from salts was occasionally encountered in the lactose region of the chromatograms, but when this occurred, deionization with pyridine (*8*) produced satisfactory results.

VALUE OF QUANTITATIVE CHROMATOGRAPHY

The quantitative chromatographic analysis of natural materials known or shown by chromatography to be composed of relatively simple mixtures of sugars, has given results comparable with those obtained by the usual chemical or fermentation methods.

In more complex mixtures, additional reducing substances have been found which were apparently calculated as lactose or total monoses in the usual sugar analysis. Occasionally, other sugars have been revealed which were not suspected of being present and were not detected by conventional sugar analysis.

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Fluorometric Determination of Zirconium in Minerals

W. C. ALFORD[1], LEONARD SHAPIRO[2], AND CHARLES E. WHITE
University of Maryland, College Park, Md.

The increasing use of zirconium in alloys and in the ceramics industry has created renewed interest in methods for its determination. It is a common constituent of many minerals, but is usually present in very small amounts. Published methods tend to be tedious, time-consuming, and uncertain as to accuracy. A new fluorometric procedure, which overcomes these objections to a large extent, is based on the blue fluorescence given by zirconium and flavonol in sulfuric acid solution. Hafnium is the only element that interferes. The sample is fused with borax glass and sodium carbonate and extracted with water. The residue is dissolved in sulfuric acid, made alkaline with sodium hydroxide to separate aluminum, and filtered. The precipitate is dissolved in sulfuric acid and electrolyzed in a Melaven cell to remove iron. Flavonol is then added and the fluorescence intensity is measured with a photofluorometer. Analysis of seven standard mineral samples shows excellent results. The method is especially useful for minerals containing less than 0.25% zirconium oxide.

AMONG the more important methods for the determination of zirconium are the gravimetric procedures involving precipitation of zirconium with cupferron (*9*), phosphate (*8*), mandelic acid (*6*), *m*-dinitrobenzoic acid (*11*), and tannin (*5*), and the colorimetric methods that make use of arsonic acids (*4*) or alizarin derivatives (*3*, *7*). None of these reagents, except mandelic acid, is claimed to be specific for zirconium, laborious separations are required, and the results obtained are not always reliable, as evidenced by the fact that different analysts often obtain widely varying results on identical samples. Mandelic acid is thought by Kumins (*6*) to be a specific precipitant for zirconium, but the amount of zirconium required for a determination makes the method unsuitable for trace analysis. Thus the need for a rapid and reliable method for estimating small quantities of zirconium prompted the development of the method described herein.

[1] Present address, National Institutes of Health, Bethesda, Md.
[2] Present address, U. S. Geological Survey, Washington 25, D. C.

The present method is based on the blue fluorescence given by zirconium and flavonol (3-hydroxyflavone) in moderately strong sulfuric acid solution when exposed to ultraviolet light. Under controlled conditions, the intensity of fluorescence is proportional to zirconium concentration. Measurements are made with a photoelectric fluorometer by comparing the intensity of fluorescence of an unknown solution with that of a known zirconium standard. The method provides for the elimination of all known interferences except hafnium and overcomes most of the objections to previous methods.

APPARATUS AND REAGENTS

Aside from the usual laboratory glass and platinum ware, the only apparatus required is a Melaven (*10*) mercury cathode electrolysis cell and a photoelectric fluorometer. Fluorescence measurements were made with a fluorometer designed and built by Alford and Daniel (*1*). During the course of this work, a Corning glass filter No. 5874 was inserted between

the Type AH-4 mercury vapor lamp and the cuvette to isolate the 3650 A. mercury line which was used to excite the zirconium-flavonol fluorescence. The wave length of the fluorescent light covers a range of 4200 to 5200 A. with a maximum at 4600 A. This was isolated with a Wratten gelatin filter No. C-5 and a Corning filter No. 3060 (to stop stray ultraviolet light) interposed between the cuvette and the photocell. The photocell was a Cetron No. CE 99 vacuum-type phototube with an S-4 spectral response.

It was determined that measurements could also be made with a Lumetron fluorescence meter, Model 402 EF, and a Beckman spectrophotometer with a fluorometric attachment as described by Fletcher *et al.* (*2*).

Standard Zirconium Solution (1 ml. = 1.00 mg. of ZrO_2). Baker's C.P. zirconyl nitrate dihydrate was dissolved in 50% sulfuric acid, twice evaporated to fumes of sulfuric acid, cooled, and diluted with water to give a solution containing approximately 10 mg. of zirconium dioxide per ml. This solution was standardized gravimetrically by precipitation of zirconium hydroxide with ammonia, followed by ignition of the hydroxide to zirconium dioxide. The appropriate dilution was then made to give a solution containing 1 mg. of zirconium dioxide per ml. in 5 *N* sulfuric acid. Immediately before use the working standard was prepared by diluting 5 ml. of the stock standard with 120 ml. of water and 375 ml. of 0.2 *N* sulfuric acid to give a solution 0.2 *N* with respect to sulfuric acid and containing 0.010 mg. of zirconium dioxide per ml. The hafnium content of the zirconyl nitrate was not known or determined, but it is probable that a small amount was present and included as zirconium.

Flavonol Solution, 0.01%. Fifty milligrams of flavonol (3-hydroxyflavone) were dissolved in 500 ml. of 95% ethyl alcohol. This solution is stable indefinitely. The flavonol was synthesized by the method of Oyamada (*12*) in the University of Maryland Organic Preparations Laboratory.

Fusion Mixture. An intimate mixture of 3 parts of anhydrous sodium carbonate plus 1 part of borax glass.

Sulfuric Acid Solutions. A 5 *N* solution was prepared by dilution and standardized as usual; 1.0 *N* and 0.2 *N* solutions were prepared, as needed, by dilution of the former.

Sodium Carbonate Solution, 2%. Twenty grams of sodium carbonate were dissolved in 1 liter of water.

Sodium Hydroxide Solution. Two hundred grams of sodium hydroxide were dissolved in distilled water, cooled, and made up to 1 liter.

EXPERIMENTAL

Before development of an analytical procedure it was necessary to study the specificity of the zirconium-flavonol reaction and the effect on the fluorescence of such variables as pH, amount of flavonol, amount of zirconium, time of standing, and temperature.

Specificity. A total of 53 common cations and anions were tested for fluorescence with flavonol in weakly alkaline, neutral, and weakly acid solutions, respectively. The ions tested were: aluminum, ammonium, antimony, barium, beryllium, cadmium, calcium, cobalt, columbium, copper, didymium (praseodymium and neodymium), gold, hafnium, iron, lanthanum, lead, lithium, mercury, nickel, platinum, potassium, selenium, sodium, strontium, tantalum, thallium, thorium, tin, titanium, uranium, zinc, zirconium, acetate, arsenate, borate, bromate, bromide, carbonate, chlorate, chloride, chromate, ferricyanide, ferrocyanide, fluoride, molybdate, nitrate, nitrite, oxalate, phosphate, silicate, sulfate, tartrate, and tungstate. Zirconium, thorium, aluminum, and hafnium gave a fluorescence in acid solution, while the tungstate ion fluoresced in neutral solution. All others were negative. Comparison of the effect of different acids (hydrochloric, nitric, sulfuric, acetic, and tartaric) showed that, in sulfuric acid, zirconium and hafnium gave strong fluorescence while aluminum was very weak and thorium was negative. Aluminum was thus found to be the only positive interference other than hafnium. About 0.250 mg. of aluminum gave a fluorescence equal to that of 0.001 mg. of zirconium.

Effect of pH. A series of 16 solutions was prepared in 25-ml. glass-stoppered cylinders. To each cylinder were added 0.010 mg. of zirconium dioxide, 1.0 ml. of 0.01% flavonol, distilled water, and varying amounts of sulfuric acid to give pH values from 0.5 to 3.4. The total volume of each solution was 25 ml., and corresponding blanks were prepared in each case. pH measurements were made with a Model ATM Macbeth pH meter. After standing for 20 minutes, the fluorescence was measured in the fluorometer. Under these conditions the optimum pH was about 1.3 (Figure 1). In practice it was found that zirconium would not long remain in solution in sulfuric acid at pH 1.3 (about 0.1 *N* sulfuric acid), so it was decided to make all measurements in 0.2 *N* sulfuric acid solution (pH 1.15 to 1.20), even though this resulted in a slightly lower sensitivity.

Concentration of Flavonol. A series of solutions of 25-ml. volume was prepared, each containing 0.050 mg. of zirconium dioxide, 0.2 *N* sulfuric acid, and amounts of 0.01% flavonol solution varying from 0 to 3.0 ml. Corresponding blanks containing no zirconium dioxide were prepared in each case. After 30 minutes' standing the fluorometer was arbitrarily set so that the sample containing 2.0 ml. of flavonol solution gave a scale reading of 100, with the corresponding blank set to read 0. Then, without changing the sensitivity of the instrument, the other solutions were measured against their corresponding blanks which were set to read 0 in each case. Results are shown in Figure 2. These data show that a minimum of 1 ml. of 0.01% flavonol solution (0.10 mg. of flavonol) is necessary for 0.050 mg. of zirconium dioxide. As the molecular weights are 238 for flavonol and 123 for zirconium dioxide, it appears fairly certain that zirconium and flavonol react on a mole:mole basis. A threefold excess of flavonol had no effect on the intensity of the fluorescence.

Zirconium Concentration. A series of 11 solutions of 25-ml. volume was prepared. Each contained 1.0 ml. of 0.01% flavonol, amounts of zirconium dioxide varying from 0 to 0.050 mg., and enough 0.2 *N* sulfuric acid to make 25 ml. After they had stood

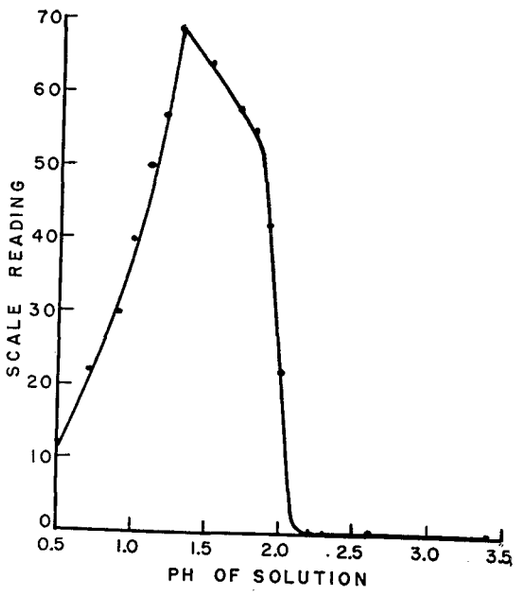

Figure 1. Effect of pH on Fluorescence Intensity

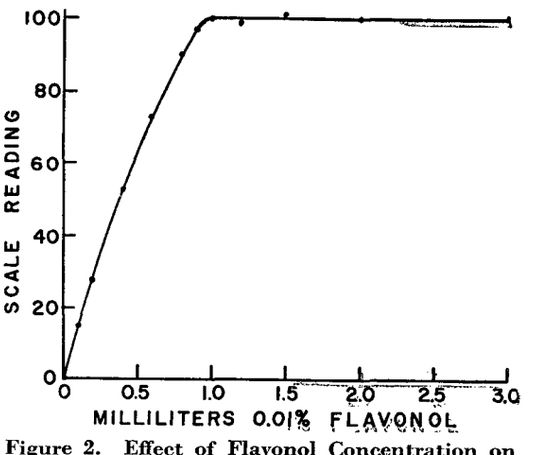

Figure 2. Effect of Flavonol Concentration on Fluorescence of 0.05 Mg. of Zirconium Dioxide

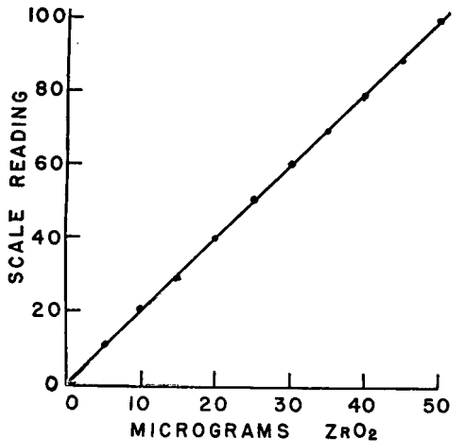

Figure 3. Relation of Zirconium Concentration to Fluorescence Intensity

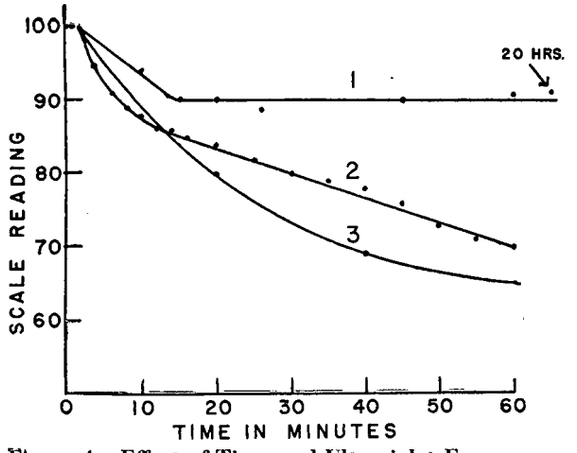

Figure 4. Effect of Time and Ultraviolet Exposure on Zirconium-Flavonol Fluorescence

1. Identical separate samples read at times shown
2. One sample read at times shown and exposed to ultraviolet only at time of readings
3. One sample exposed to ultraviolet continuously

30 minutes, the fluorometer was set so that the blank read 0 and the 0.050-mg. standard read 100. The remaining solutions were then measured. Results are shown in Figure 3.

The fluorescence intensity is a linear function of zirconium concentration over the 0 to 0.050 mg. of zirconium dioxide range. The sensitivity of the fluorometer would not permit extension of the curve to higher concentrations of zirconium. Three additional experiments were carried out in exactly the same manner, except that the ranges of zirconium dioxide concentrations were 0 to 0.005, 0 to 0.010, and 0 to 0.020 mg. per 25 ml., respectively. In each series the solution of greatest zirconium content was set to read 100 by appropriate adjustment of fluorometer sensitivity settings, and in each case fluorescence intensity was proportional to zirconium concentration.

Effect of Time of Standing on Fluorescence. Three experiments were conducted, with the results shown in Figure 4.

Curve 1 represents a series of seven identical solutions each containing 0.010 mg. of zirconium dioxide, 1 ml. of 0.01% flavonol, and 0.2 *N* sulfuric acid to make a volume of 25 ml. The fluorometer was set to give a scale reading of 100 about 2 minutes after mixing the solutions, and the other six solutions were measured at the time intervals indicated. Each sample was discarded after being measured. Curve 2 represents one solution, identical with the above, which was measured at the intervals shown. It was exposed to ultraviolet light only while readings were being taken. Curve 3 shows the effect of time and continuous exposure to ultraviolet light over a period of 1 hour.

These data show that exposure to ultraviolet light results in a lowering of fluorescence intensity. In the absence of ultraviolet light exposure, the intensity of fluorescence diminishes rather rapidly for about 15 minutes and is then stable for at least 20 hours.

Effect of Temperature. Normal variations in room temperature had little effect on the intensity of the zirconium fluorescence, and in hot solutions (80° to 100° C.) the fluorescence was destroyed but slowly returned upon cooling. Because the proposed method makes use of a standard zirconium solution for every determination, any possible temperature effect is automatically compensated.

The optimum conditions thus determined for pure solutions were: 25 ml. of solution, 0.2 *N* with respect to sulfuric acid containing 0 to 0.050 mg. of zirconium dioxide and 1 ml. of 0.01% flavonol, mixing at room temperature, and standing for at least 20 minutes before measuring.

Negative Interferences. A systematic study of the effect of other ions on the zirconium fluorescence showed that, of the common ions, only ferric iron, fluoride, and phosphate had any quenching effect. Even trace quantities of these ions either destroyed or greatly diminished the fluorescence and must be removed. The authors are investigating the possibility of using this reaction to determine fluoride and phosphate ions. The alkali and alkaline earth metals, ferrous iron, titanium, zinc, tin, thorium, citrates, tartrates, acetates, borates, chlorides, and nitrates were found to have no effect, except that high concentrations of any salt caused a quenching effect which increased with concentration. Strong oxidizing agents, substances which precipitate (silicon dioxide, etc.), and highly colored ions must be absent. The procedure described below eliminates all the known interferences both positive and negative, except hafnium.

Analytical Method for Clays, Sands, and Refractories. Weigh out a sample of powdered and dried mineral estimated to contain from 0.050 to 0.250 mg. of zirconium dioxide. Place a quantity of the fusion mixture about 10 to 15 times the weight of the sample in a medium-sized platinum crucible and fuse over a Meker burner. Cool and place the sample on top of the melt. Cover and carefully fuse again for 15 to 20 minutes over a Meker burner or in a muffle furnace at 900° to 1000° C. Gently swirl the contents of the crucible two or three times during the course of the fusion. Cool slightly and then immerse the crucible to one half its depth in 100 ml. of distilled water in a 400-ml. borosilicate glass beaker. This helps to crack the melt as it cools and speeds up solution later. Drop the crucible into the water, cover with a watch glass, boil for a few minutes, and then digest on the steam bath until the melt is thoroughly disintegrated (an overnight period is convenient). Break up any lumps with the aid of a flat-end stirring rod. Remove the crucible and wash any adhering particles into the beaker.

Bring to a boil again, stir vigorously to break up the precipitate, cool slightly, and filter through a 9-cm. Whatman No. 40 paper (or its equivalent). Wash the beaker and precipitate two or three times with 2% sodium carbonate solution and twice with water. Discard the filtrate and place the original beaker under the filter. Dissolve the precipitate by the dropwise addition of 5 *N* sulfuric acid.

When all precipitate is dissolved, wash thoroughly with water. Make the filtrate alkaline to phenolphthalein with 5 *N* sodium hydroxide, then add a 5-ml. excess of the alkali. Stir, let stand for a few minutes, and filter, using the same paper employed in the first filtration. Wash five or six times with water, discard the filtrate, and place a 100-ml. volumetric flask under the filter. Dissolve the precipitate with exactly 20 ml. of 1.00 *N* sulfuric acid, added dropwise in two portions from a 10-ml. pipet. Wash the paper thoroughly with water and dilute the filtrate to 100 ml. with distilled water. Pipet a 10-ml. aliquot into the Melaven cell and electrolyze for 45 minutes at a current of about 0.5 ampere to remove iron and any other heavy metals. Drain into a 25-ml. glass-stoppered cylinder and wash the cell with about 10 to 12 ml. of 0.2 *N* sulfuric acid. Add 1 ml. of 0.01% flavanol, make up to 25 ml. with 0.2 *N* sulfuric acid, mix, and let stand for 20 minutes out of direct sunlight. At the same time, prepare a reagent blank and a standard zirconium dioxide solution, each containing 1 ml. of 0.01% flavonol and 0.2 *N* sulfuric acid to make a volume of 25 ml.

The amount of zirconium dioxide in the standard may be from 0.010 to 0.050 mg., depending on the quantity in the sample. In

the course of the present work the fluorometer was set so that the blank and standard read 0 and 100, respectively. In the more general case where this is not possible, the blank and standard readings are plotted on straight graph paper and the amount of zirconium dioxide in the unknown is obtained by reference to the calibration curve thus obtained. It is necessary to establish the linearity of the instrument used under the above experimental conditions.

Calculate the percentage zirconium dioxide as follows:

$$\frac{\text{Mg. of } ZrO_2 \text{ in standard}}{(S - B)} \times (U - B) \times \frac{\text{aliquot factor} \times 100}{\text{sample wt. (mg.)}} = \%\ ZrO_2 \text{ in mineral}$$

where S, U, and B = scale reading of standard, unknown, and blank, respectively.

EXAMPLE. An aliquot representing one tenth of a 200.0-mg. sample reads 55 against a blank that reads 30 and a 0.010-mg. zirconium dioxide standard that reads 80. The zirconium dioxide in the mineral is then

$$\frac{0.010}{(80 - 30)} \times (55 - 30) \times \frac{10 \times 100}{200.0} = 0.025\%$$

Discussion. Preliminary extraction of the fused mineral with water alone results in the separation of chlorides, fluorides, sulfates, phosphates, thorium, most of the silica, part of the aluminum, and other metals which form soluble ions in an alkaline carbonate medium. The sodium hydroxide treatment removes aluminum along with any remaining alkali-soluble ions, and mercury cathode electrolysis removes all iron and other heavy metals such as copper. The sodium hydroxide precipitate must be formed in the cold, filtered, and dissolved within 1 to 2 hours, because under the influence of heat or long standing the titanium precipitate becomes insoluble in dilute acids. The separations provided are sufficiently clean to permit accurate measurement of the zirconium content of the final solution.

Of the minerals analyzed, only one gave any difficulty in the above procedure. In analyzing Bureau of Standards sample 91 (opal glass) containing 10.5% calcium oxide it was found that the sodium hydroxide precipitate would not dissolve readily in 1 N sulfuric acid. In this case the paper and precipitate were treated with sulfuric and hydrofluoric acids in a platinum dish, evaporated to dryness, heated to ash the paper, and fused at low heat with 2 grams of potassium pyrosulfate, and the melt was dissolved in 0.2 N sulfuric acid to give 100 ml. of solution. The remainder of the procedure was the same as usual, except that the standard and blank contained 2% potassium pyrosulfate to equalize salt concentrations.

It was found that good results could be obtained on glass sands (low in iron and aluminum) by a simple procedure involving a sulfuric acid–hydrofluoric acid treatment to remove silica, fusion of the residue with potassium pyrosulfate, and direct determination of the zirconium content in the sulfuric acid solution of the melt. This procedure is not recommended, because even trace quantities of iron cause low results and should be removed in all cases.

The flavonol reagent exhibits a moderately strong green fluorescence when exposed to ultraviolet light. Fortunately, this fluorescence is sufficiently different from that of the zirconium-flavonol complex to permit isolation of the desired light by a judicious choice of filters between the cuvette and phototube. Of the many combinations tested, the one suggested (Wratten gelatin filter No. C-5 and Corning filter No. 3060) was found most appropriate. This system filters out the flavonol fluorescence almost completely without appreciably interfering with the passage of the blue-white light produced by the zirconium-flavonol complex. Thus, under the conditions given, the self-fluorescence of the flavonol is a negligible factor. It must be emphasized that this filter system, or its equivalent, is essential to the successful use of the method as outlined.

As indicated above, hafnium gives a fluorescence with flavonol. A standard solution of hafnium was prepared by fusing hafnium dioxide with potassium pyrosulfate and dissolving the melt in 0.2 N sulfuric acid. Upon visual comparison of the hafnium and zirconium fluorescence, no qualitative difference could be detected. Quantitative comparison, on a mole:mole basis, showed that equivalent quantities of hafnium and zirconium give approximately equal fluorescence with flavonol. As there is no feasible means for separating small amounts of hafnium and zirconium, the present method is actually for the determination of zirconium and hafnium combined. In this respect, this method does not differ from other analytical methods for zirconium.

Analytical Results. The method was tested on seven standard samples from the Bureau of Standards, which included burnt refractories Nos. 76, 77, and 78, glass sand No. 81, opal glass No. 91, and clays Nos. 97 and 98. The zirconium dioxide content of these minerals varied from 0.0095 to 0.25%. Table I shows the results of these analyses and the average values for zirconium dioxide content as given in the Bureau of Standards certificates of analysis. Such average values represent the work of bureau analysts as well as that of competent cooperative analysts of other institutions.

Table I. Determination of Zirconium Dioxide

Standard Sample No.	Type of Mineral	Per Cent ZrO_2: Bureau of Standards certified value	Per Cent ZrO_2: Found by flavonol method
76	Burnt refractory	0.07	0.070
77	Burnt refractory	0.09	0.092
78	Burnt refractory	0.12	0.117
81	Glass sand	0.031	0.032
91	Opal glass	0.0095	0.0092
97	Flint clay	0.25	0.253
98	Plastic clay	0.041	0.043

The values found by the fluorometric method vary from the average values given by the National Bureau of Standards by a maximum of about 5%. The maximum variation between results of different analysts, as given by the Bureau of Standards certificates of analysis, was up to 50% or more in some cases—for example, values of from 0.032 to 0.050% zirconium dioxide were reported for sample 98, and from 0.009 to 0.039% for sample 81. Green (*3*) reported a zirconium dioxide content of 0.025% in sample 98; however, the above results indicate that the Bureau of Standards value is correct. Since the average of the figures reported is accepted as being close to the true value, the fluorometric method may be considered to be very reliable and probably gives more accurate results than the best gravimetric procedures when the zirconium content is below 0.10%.

ACKNOWLEDGMENT

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Determination of Organic Nitrogen

Control of Variables in the Use of Nessler's Reagent

JOHN F. THOMPSON[1] AND GEORGE R. MORRISON
Botany Department, University of Rochester, Rochester, N. Y.

In the analysis of plant samples containing a small quantity (5 to 200 micrograms) of nitrogen, it was essential to have an accurate method reproducible from day to day. The colorimetric nesslerization method developed makes possible reproducible results by the careful control of variables. The most important variables are the preparation and maintenance of the Nessler's reagent solution, the conditions during digestion of the sample, the temperature of the sample during nesslerization, and the alkalinity of the sample before and after nesslerization. Where interfering substances cause turbidity, the ammonia can be recovered and determined by addition of excess potassium iodide and distillation. It is possible to analyze samples without running a standard curve with each set. Any sample can be analyzed, even though it contains substances which interfere in ordinary nesslerization procedures.

IN RECENT investigations on nitrogen metabolism in plants, it has been necessary to determine amino nitrogen. To determine all reduced nitrogen compounds, the organic matter is digested with sulfuric acid, forming ammonium sulfate. For relatively large amounts of nitrogen (0.5 mg. and up) the Kjeldahl semimicromethod (in which the ammonia is distilled off from an alkaline solution, collected, and determined by titration) is excellent. In the determination of smaller quantities (5 to 200 micrograms of nitrogen), the nesslerization procedure has been found useful because distillation is not necessary and the only apparatus required is a colorimeter.

VARIABLES AND PROCEDURE FOR DETERMINATION OF ORGANIC NITROGEN

The use of Nessler's reagent for the determination of microgram quantities of ammonia nitrogen is well established. However, the nesslerization method is accurate only if a number of conditions are carefully controlled. The colored complex (NH_2-Hg_2I_3) formed from ammonia and Nessler's reagent (K_2HgI_4) is very insoluble in water and therefore forms only colloidal solutions (*9, 13*). After all organic compounds are converted to ammonia, the determination of nitrogen by measurement of color using Nessler's reagent may be erratic because of the numerous factors—e.g., pH, temperature, salts, method of formation, speed of formation, charge on particles, etc.—to which colloidal solutions are especially sensitive.

These factors not only may cause turbidity but will affect the intensity of color even when the solutions are clear.

The variables in the Nessler method have been recognized previously, but to obtain reproducible results they must be more thoroughly controlled than hitherto. They include:

Preparation of Nessler's solution (*5, 12, 13*)
Condition of Nessler's reagent at the time of nesslerization (*12, 13*)
Digestion of sample (*5, 8, 10*)
Temperature of sample during nesslerization (*8, 10*)
Time of reading the color produced after nesslerization (*1, 8, 10*)
Rate of addition of Nessler's reagent and rate of mixing (*8*)
Alkalinity of solution before and after nesslerization (*5, 6, 12, 13*)
Extraneous materials such as salts, metals, and organic compounds (*2, 5, 8, 11*)

These factors have been reconsidered in the method now presented and are discussed in some detail herewith.

[1] Present address, Botany Department, Cornell University, Ithaca, N. Y.

REAGENTS

1. Standard Ammonium Sulfate Solution. Solutions of ammonium sulfate containing 25, 50, 75, and 100 micrograms of nitrogen per 10 ml.
2. Approximately 3.0 *N* Sulfuric Acid (8 ml. of concentrated sulfuric acid per 100 ml.). Five milliliters of this solution are sufficient for most samples containing 150 micrograms of nitrogen or less.
3. Hydrogen Peroxide, 30% solution. This should be free of nitrogen (for Procedure 1).
4. Digestion Mixture. Potassium sulfate–copper sulfate 3 to 1 (for Procedure 2).
5. Sodium Hydroxide, 5% or 1.25 *N*. The solution is allowed to stand several days and filtered. Any precipitate which forms will cause turbidity of nesslerized solution.
6. "Restoration Solution." Potassium iodide (7.5 grams) plus iodine (5.6 grams) per 100 ml. of water.
7. Nessler's Reagent Solution. The preparation is based on Koch and McMeekin's modification of Folin's Nessler's reagent (*5*). Thirty grams of potassium iodide and 22.4 grams of iodine are dissolved in 200 ml. of distilled water, and 30 grams of pure mercury are added. The solution is stirred constantly (several hours) until its color changes from reddish brown to a light yellowish orange. (No metals should be allowed to come in contact with the solution, because they will cause turbidity in the final nesslerized solution.)

Nessler's solution as commonly understood consists of a mixture of Nessler's reagent solution (reagent 7) and sodium hydroxide solution (reagent 5).

As soon as this color intensity is reached, the solution is filtered through a sintered-glass funnel to remove excess mercury. Use of filter paper has frequently resulted in slight turbidity of nesslerized ammonia solution. To be effective for use, the Nessler's solution should give a faint starch test for iodine and give a colorimeter reading of 300 to 500 on a Klett colorimeter, in a 1-cm. Klett tube with Corning filters 3389 and 5113 of standard thickness. All subsequent readings in this paper were made in this manner.

If the colorimeter reading is less than 300, the color will be greenish yellow, there may be no starch test, and mercury may be in excess, causing marked turbidity in the final nesslerized solution (especially at higher concentrations of nitrogen). This condition can be completely remedied by the addition of the restoration solution; 0.1 ml. of restoration solution increases the colorimeter reading of 100 ml. of Nessler's solution approximately 70 units.

When the colorimeter reading of the Nessler's reagent solution is above 500, potassium iodide (KI_3) is in excess and may result in reduced color production with ammonia. In this case, it is necessary to mix the solution with more mercury.

The Nessler's reagent solution may be satisfactory for use if its density as shown by the colorimeter reading is as low as 200 or as high as 700. However, the solution should be tested first with 100 micrograms of nitrogen for both turbidity and color development.

The Nessler's reagent solution may, after several weeks, decompose to the point where its colorimeter reading is below 300;

therefore it should be tested colorimetrically every week. In case of decomposition, addition of restoration solution will bring the solution back to normal.

DIGESTION AND PREPARATION OF SAMPLE OF NESSLERIZATION

Because many factors such as salts, metals, etc., other than poor Nessler's solution will result in turbid nesslerized solutions, three alternative procedures are necessary. These alternatives take account of and allow for the risk of turbidity in the first solutions.

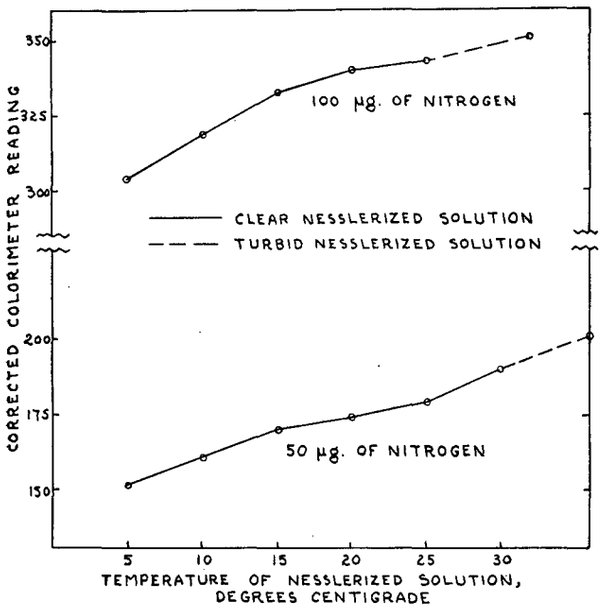

Figure 1. Effect of Temperature of Solution on Amount of Color Produced and Turbidity

Ammonium sulfate treated with Nessler's reagent solution and adjusted to desired temperature before addition of sodium hydroxide. Color measured 20 minutes after addition of sodium hydroxide solution

Procedure 1. This is used when turbidity in the nesslerized solutions is not expected.

The sample containing organic nitrogen is placed in a small (100-ml.) Kjeldahl flask, and to it are added a glass bead and 4 ml. of 3 *N* sulfuric acid. The flask is placed over an open flame at a 45° angle and heated until the volume is reduced to approximately 1 ml. The boiling should be watched so that the bead bounces continually, thus preventing any loss by bumping. The bead may be kept moving by tapping the flask.

When the volume is less than 1 ml. and faint white fumes appear, digestion of the sample has begun. During digestion, the flame is reduced so that the sulfuric acid condenses about half-way up the bulb of the Kjeldahl flask, but does not pass into the neck of the flask, and there is always liquid around the bead. The sample is heated for 10 minutes under these conditions and then cooled to room temperature. Two drops of hydrogen peroxide are added and heated for 2 minutes as above. If the sample is not yet clear, the addition of hydrogen peroxide to the cooled sample and the 2-minute heating are repeated. This procedure is repeated until the solution is finally clear.

When the sample is clear and cool, it is washed into a 25-ml. volumetric flask with 15 ml. of water or less, and 5% sodium hydroxide is added to the volumetric flask, 1 ml. at a time, until the solution is neutral or basic as tested with a very small volume of solution on litmus paper. (Unless the determinations are to be completed promptly, the digested solutions should remain acid.) More than 1-ml. excess of 5% sodium hydroxide solution will cause turbidity when the solution is treated with Nessler's reagent solution.

The alkaline solution is made up to volume (25 ml.) and 20 ml. are removed to a 50-ml. test tube (2.0 cm. in diameter) for the nesslerization procedure.

Procedure 2. This is used when turbidity due to salts, metals, etc., is expected.

The sample containing organic nitrogen is placed in a 125-ml. Kjeldahl flask with 10 ml. of 3.0 *N* sulfuric acid, 20 mg. of the digestion mixture (reagent 4), and a glass bead. The solution is evaporated to about 1 ml. and digested as in Procedure 1. When the clear, digested sample has cooled to room temperature, 20 ml. of 5% sodium hydroxide are added and the flask is immediately connected to a standard type of micro-Kjeldahl distillation apparatus. The outlet of the condenser is inserted into a 50-ml. volumetric flask under the surface of 4 ml. of 0.3 *N* sulfuric acid. The Kjeldahl flask is now heated until about 30 ml. of water have distilled over into the volumetric flask, 1 ml. of 5% sodium hydroxide is added to the flask, and the sample is made up to volume. Then 20 ml. of this solution are pipetted into a 50-ml. test tube for the nesslerization procedure.

Procedure 3. This is used when turbidity has resulted from nesslerization carried out in Procedure 1.

The turbid nesslerized solution is washed into a 125-ml. Kjeldahl flask with 25 ml. of distilled water and a glass bead is added. Potassium iodide crystals are added in excess until the turbid orange color becomes clear and gives place to a faint yellow coloration. An excess of potassium iodide will ensure the release of all the nitrogen in the form of ammonia.

The flask is connected to a Kjeldahl-type distillation apparatus and the distillation procedure is carried out as in Procedure 2. The solutions are now ready for the nesslerization procedure.

When a sample is low in both oxidizable material and nitrogen, it is advisable to reduce the quantity of sulfuric used for digestion, neutralize the acid, and nesslerize the entire sample directly in the tube used for the digestion. In this way, the final volume is kept small, thus increasing the sensitivity.

NESSLERIZATION PROCEDURE

The 20-ml. aliquots of the solution resulting from the various methods described above are brought to 20° C. in 50-ml. test tubes in a water bath. Air is bubbled through at a rapid rate, but not so fast that the solution will overflow when the Nessler's reagent solution and sodium hydroxide solutions are added. Then 1 ml. of Nessler's solution is run down the side of the tube, followed by 5 ml. of 5% sodium hydroxide. The rate of addition of the base is not critical, if the mixing is thorough.

The air jet is now removed. It is not necessary that the nesslerized solution remain at 20° C. after the addition of the base. The color is determined exactly 20 minutes after addition of the sodium hydroxide, with a colorimeter or spectrophotometer

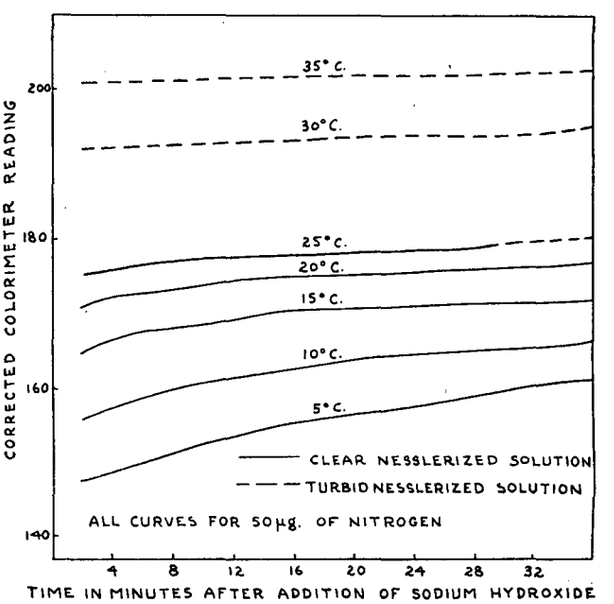

Figure 2. Effect of Time and Temperature on Amount of Color and Turbidity in Nesslerized Ammonia Solution

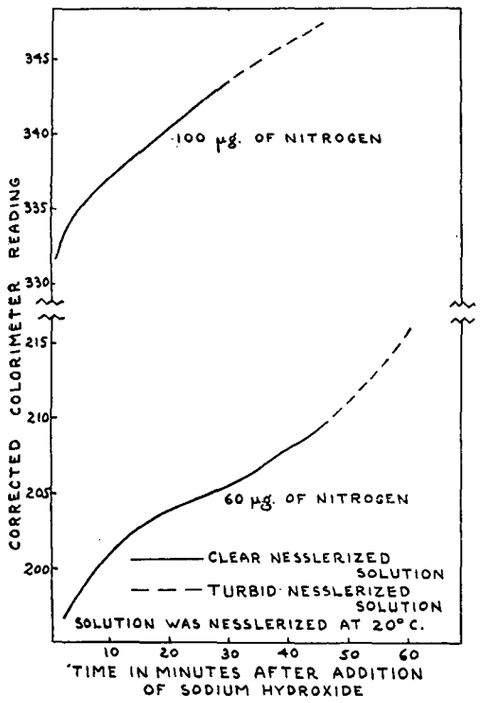

Figure 3. Effect of Time on Amount of Color and Turbidity

so arranged that the light beam has a peak at about 420 mμ. A Klett colorimeter with Corning filters 3389 and 5113 (standard thickness) has been found satisfactory.

Blank and standard samples should be run through precisely the same procedure. The standard curve (relating the colorimeter readings corresponding to 25, 50, 75, and 100 micrograms of nitrogen) is plotted after making the appropriate blank correction to the colorimeter readings. The nitrogen in the nesslerized aliquot of an unknown sample is that which corresponds on the standard curve to the corrected colorimeter reading. The nitrogen in the original digested sample can then be readily calculated.

Various factors have been found to influence the final result.

Nessler's Solution. Nessler's solutions prepared from mercuric iodide rather than metallic mercury gave turbid solutions. A Nessler's solution containing gum ghatti made by the method of Johnson (*3*) was found unsatisfactory because of the limited amount (50 micrograms) of nitrogen that could be analyzed.

The Nessler's reagent solution is stable if prepared as described (reagent 7). Short periods (2 hours) at 80° C. or even bubbling air through the Nessler's solution for 12 hours at room temperature did not decompose it.

The addition of excess sodium hydroxide to a digested sample after neutralization but before addition of the potassium mercuric iodide (Nessler's) solution will result in turbidity. The customary Nessler's solution which contains both potassium mercuric iodide and sodium hydroxide is more convenient to use, but this is inadvisable because a slight precipitate forms after a few hours' standing and must be removed by filtration before the reagent is utilized. In addition, the length of time when a Nessler's solution can produce maximal reproducible color with ammonia is much shorter when the sodium hydroxide and potassium mercuric iodide solutions are combined. It is essential therefore to store the alkali (reagent 5) and the Nessler's reagent solution (No. 7) separately.

Digestion of Organic Matter. Digestion of organic matter is of primary importance in obtaining accurate determination of nitrogen (*3*, *5*, *7*). The details of the digestion procedure are often an individual matter, depending on the material to be analyzed.

In the analysis of primary amino compounds, principally amino acids, copper is a satisfactory catalyst, although mercury may be preferable for other nitrogen compounds (*4*). Loss of nitrogen during digestion depends directly on the temperature at which the sample is digested and the length of time the sample is digested. Loss of nitrogen will be marked unless liquid sulfuric acid is kept in the bottom of the digestion flask at all times during the digestion. If the heat is adjusted so that the sample is digested within an hour, the loss will be less than 1%.

Temperature of Solution During Nesslerization. Variations in temperature during the actual process of nesslerization have a marked effect on turbidity and the final intensity of color developed (*8*, *10*). They also influence the rate of color development. Figure 1 gives the effect of temperature on final color development for 50 and 100 micrograms of nitrogen. As the temperature of nesslerization increases, the color increases. A 10° C. change in temperature will alter the amount of nitrogen recovered by about 9% with nonturbid solutions. That the effect of temperature is not just due to its effect on the rate of reaction with ammonia is shown in Figure 2. At lower temperatures, the color never reaches that obtained at higher temperature, even if no turbidity results. The temperature, therefore, has a specific effect on the final color, and on how quickly the solutions will become turbid. The higher the temperature, the more rapid is the appearance of turbidity.

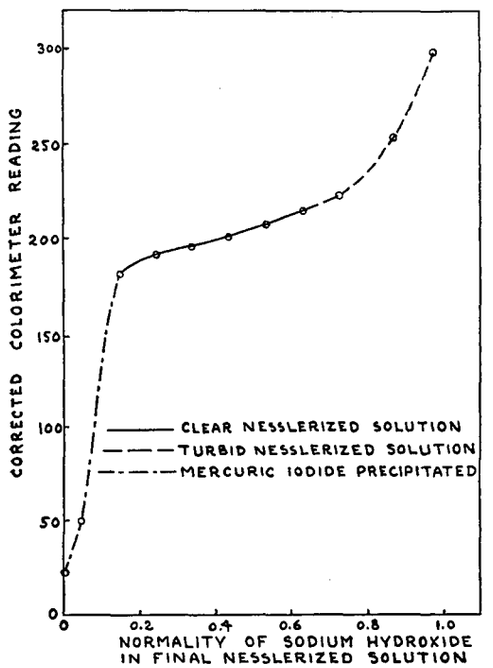

Figure 4. Effect of Final Sodium Hydroxide Concentration on Color and Turbidity

Nesslerized ammonia solution containing 50 micrograms of nitrogen

In these circumstances it was decided to carry out the nesslerization procedure at 20° C., safely below the temperature that produces turbidity in 20 minutes even with 100 micrograms of nitrogen (Figure 3). The intensity of color produced at 20° C. is relatively high and relatively constant with time (Figure 2).

Alkalinity of Solution During Nesslerization. The concentration of sodium hydroxide before and after nesslerization is critical (*5*, *6*, *12*, *13*). If the sodium hydroxide concentration in the final nesslerized solution is less than approximately 0.15 *N*, mercuric iodide [according to Leitch (*6*), the red precipitate is mercuric oxide] precipitates (*6*) and color formation is incomplete (*5*) (see Figure 4). If the concentration of base is too high (above

0.6 N) the solution becomes turbid. However, even in the intermediate range where mercuric iodide does not precipitate and the solution remains clear, the concentration of sodium hydroxide has a considerable effect on color development. In the stated procedure, the final sodium hydroxide concentration is about 0.25 to 0.30 N. Mercuric iodide may precipitate even when the concentration of base is more than 0.15 N. This occurs when there are a relatively high concentration of sodium sulfate and a relatively low concentration of ammonia in the solution. However, color development is unaffected by the precipitation of mercuric iodide per se (see Figure 4). A 0.40 N increase in the final sodium hydroxide concentration increases the apparent nitrogen recovered by about 1%. For this reason, all digested samples are neutralized and then an appropriate and constant excess of base is added, so that the final hydroxide concentration is always the same.

In the procedure prescribed, samples are neutralized carefully before the addition of an appropriate constant quantity of sodium hydroxide. There are several reasons for keeping the final sodium hydroxide concentration constant. The color developed varies with the final hydroxide ion concentration in the solution; considering the effect of temperature on color development, the resulting heat of neutralization and heat of dilution on temperature will alter the final color production; this procedure obviates the need for using a high concentration of sodium hydroxide where the region between mercuric iodide precipitation and turbidity is narrow if large amounts of sulfuric acid have been used for digestion.

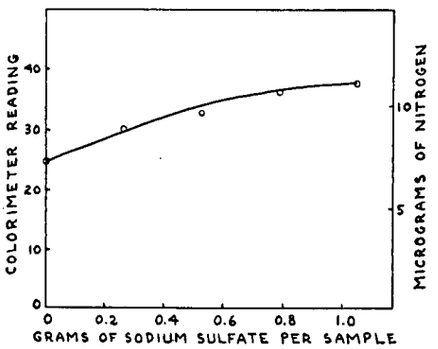

Figure 5. Effect of Quantity of Sodium Sulfate on Amount of Color Produced in Nesslerized Blank Solution

Amount of Organic Matter Digested and Presence of Sulfate in Final Solution. A small error will result if the quantity of oxidizable material in the sample varies widely, as sodium sulfate increases the blank color (Figure 5). Because the curve is not linear, the effect of the sodium sulfate cannot be due to ammonia impurities. Furthermore, as the amount of sodium sulfate increases, the tendency toward turbidity is increased, especially at high nitrogen concentrations. After digestion, the amount of sulfuric acid remaining in the sample will vary inversely as the quantity of oxidized material.

Effect of Time After Nesslerization on Color Density. The time after nesslerization when colorimeter readings are made is important and should be constant. This time interval must be chosen so that the solution is not turbid even with 100 micrograms of nitrogen and so that the further change in color with time is minimal. Figure 3 indicates that the chance of encountering turbidity is greater with the lapse of time and that turbidity after nesslerization will appear sooner with samples containing greater amounts of nitrogen. At 20° C. even with 100 micrograms of nitrogen, there is no turbidity in 20 minutes. However, the appearance of turbidity also depends on final hydroxide concentration. Therefore, the choice of 20 minutes after nesslerization as the time when the colorimeter readings should be made was based on the following considerations: After 20 minutes the intensity of color developed varies but little with time (Figure 3). Even with 100 micrograms of nitrogen no turbidity develops at 20° C. in 20 minutes. A variation of ±1 minute from 20 minutes alters the nitrogen value as determined by less than 0.01%. Hoffman *et al.* (*1*) made their colorimeter readings 2 minutes after nesslerization. Figure 3 shows how undesirable this is because of the rapid change of color with time at 2 minutes. Figure 3 agrees well with the data of Miller and Miller (*8*).

Table I. Comparison of Nitrogen Analyses on Four Samples

Sample[a] No.	Nitrogen, Mg. per Ml. of Sample: Kjeldahl method	Nessler's method	% Difference
1	1.66	1.67	0.6
2	1.06	1.00	6.0
3	0.64	0.65	1.5
4	1.50	1.44	3.3

[a] Samples 1 and 3 were potato extracts. Samples 2 and 4 were hydrolyzates of potato.

Table II. Comparison of Nitrogen Analyses on Two Samples by Nesslerization Method

(Alternative procedures 1 and 3 of this paper)

Sample[a]	Nitrogen, Micrograms per Ml. of Sample: Procedure 1	Procedure 3	% Difference
A	32.1	32.2	0.3
B	31.9	32.8	2.8

[a] Hydrolyzed protein solutions.

Time Interval Between Addition of Nessler's Reagent and of Alkali. The time interval between the addition of Nessler's reagent (solution 7) and the excess of sodium hydroxide is not important. As it increases, mercuric iodide will precipitate, but this does not affect the final color production.

Rate of Mixing During Nesslerization. The rate at which the reactants are mixed, while the Nessler's solution is being added, has no effect on color produced. However, the rate of mixing while the sodium hydroxide is being added—i.e., while the color is being developed—is extremely important in determining the final color. Localization of sodium hydroxide due to inadequate mixing causes turbidity, and thorough mixing of the solutions during the addition of the sodium hydroxide will prevent this.

Standardization of Nesslerization Procedure. It is not necessary to make a standard curve with every set of samples. The procedure prescribed will give standard curves which follow Beer's law and do not vary from day to day. Between six standard curves obtained on different days, the standard deviation was less than 2%. Consequently, for these conditions one standard curve may be used at all times. It is desirable, however, to use one standard sample with each batch as a general check on the procedure.

Range and Sensitivity of Method. As given above, the method is satisfactory with amounts of nitrogen up to 100 micrograms. It can be used satisfactorily up to 150 micrograms of nitrogen when no interfering substances are present to cause turbidity.

Interfering Substances. Under Procedure 2, there are no complications from interfering materials. Under Procedure 1 many salts will interfere either to cause turbidity or to change color intensity (*5, 8, 11, 12*). Metals that react with iodine will always result in turbidity. Acetone in the solution to be nesslerized in extremely small amounts will prevent color formation (*2*). Ethyl alcohol in the solution to be nesslerized will cause turbidity, but only at much higher concentrations.

RESULTS USING NESSLER'S METHOD AS PRESCRIBED

In order to test the method, organic nitrogen was determined by both the Nessler method and the conventional Kjeldahl method in

which the resultant ammonia is distilled, absorbed in acid, and determined by titration. Table I shows that the average difference between the two methods was 2.8%.

Results using Procedure 3 agree well with those with Procedure 1 (Table II).

SUMMARY

In the nesslerization procedure described careful control of conditions makes it unnecessary to run a standard curve more than once.

Many factors in the nesslerization procedure may cause incomplete color development, inconsistent color development, or turbidity: type of Nessler's solution; digestion; temperature and alkalinity of solution to be nesslerized; amount of sodium sulfate; time interval between addition of base and making of colorimeter reading; and rate of mixing during addition of sodium hydroxide. The proper recognition and control of these factors make the prescribed procedure quantitatively reproducible from day to day.

The use of two solutions (potassium mercuric iodide and sodium hydroxide) is introduced.

An alternative procedure, presented in detail, will give accurate results, complete color development, and no turbidity even when the nesslerized sample gives unsatisfactory results by the ordinary nesslerization procedure. A sample that has been found unsatisfactory can be analyzed by this procedure.

ACKNOWLEDGMENT

This work was applicable to the general purposes of research, being carried out in the Botany Department of the University of Rochester under grants from the Growth Committee of the National Research Council and the National Cancer Institute to F. C. Steward. Technical assistance for this work was furnished from these grants and grateful acknowledgment is here made for this help.

The authors greatly appreciate the encouragement and help of F. C. Steward.

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Syringe-Type Apparatus for Quantitative Hydrogenation

At Constant Pressures Above 1 Atmosphere

C. W. GOULD AND HERBERT J. DRAKE

General Aniline & Film Corp., Easton, Pa.

The need in this laboratory for catalytic hydrogenolysis of 0.2- to 2.0-gram samples of azo dyes, nitro compounds, etc., led to the development and routine use of an apparatus wherein a metal syringe replaces the conventional mercury piston as a means of restoring the initial pressure. Pressures up to 5 atmospheres are used for reductions requiring 0.5 to 12.0 millimoles of hydrogen. The standard deviation of the per cent relative error is 0.7 for a single determination. No correction is necessary for solvent vapor pressure.

THERE is described below a rugged hydrogenation apparatus which in 2 years' use has proved convenient and accurate for samples requiring 0.5 to 12 millimoles of gas.

APPARATUS

Figure 1 shows that the arrangement is like many described previously (*1–4*, *6*), except that a metal syringe replaces the double manometer and mercury leveling bulb, a sensitive Bourdon gage serves as the pressure indicator, and a long stainless steel capillary is used to connect the reactor with the measuring system.

Figure 2 shows the arrangement of the component parts.

Valves and Manifold. Connections are made with fittings having the standard 1/8-inch pipe thread. Lunkenheimer stainless steel needle valves are installed to resist leakage of hydrogen from the manifold.

Syringe. The syringe consists of a water-jacketed cylinder 7 inches (17.5 cm.) long and 1.180 inches in bore, and a piston moved by a screw 18 threads per inch and 0.625 inch in diameter. Each turn of the screw displaces 1.00 ml. On the circumference of a wheel attached to the screw are engraved ten divisions, each equivalent to 0.10 ml. A sectional drawing (Figure 3) shows the construction of the syringe. The cord packing in the piston is clamped tightly between washers held by a cap bolt. To prevent leakage of hydrogen through and around this packing, a thick mixture of gear oil (Texaco Thuban 250) and fine graphite provides a satisfactory seal. No leakage of hydrogen at 5 atmospheres can be detected in 24 hours.

Gage. An Ashcroft laboratory test gage, 0 to 120 pounds per square inch in 0.5 pound per square inch divisions, was calibrated up to 5 atmospheres with a 10-foot open mercury manometer, and a new dial was made to read directly in absolute atmospheres. Any deviation from 1 atmosphere caused by barometric change is applied as a correction to the initial and final gage readings.

Connection Between Manifold and Reactor. As the reactor is rocked by a shaker, a flexible connection is necessary. This is a 10-foot length of stainless steel tubing, 0.125 inch in outside diameter (1.25 mm. in bore), with two coiled sections as shown in Figure 2.

This tube is important in preventing solvent vapors from diffusing back into the syringe and gage.

Reactor. The reactor was made by the Corning Glass Works from standard heavy-walled 1-inch pipe according to the dimen-

sions in Figure 4. The open end is the regular Corning borosilicate glass pipe ending.

The reactor is connected to the connecting tube by bolting the standard borosilicate glass fitting to a flange brazed to the connecting tube.

From a hook on the inlet tube hangs a sample cup made from a lipless 5-ml. borosilicate glass beaker. The inlet tube opens to the side, rather than to the bottom, in order to avoid blowing material from the sample cup when hydrogen enters the reactor.

The gasket is of two layers: Next to the glass face is a layer of 0.125-inch Teflon; next to the Teflon is smooth $^1/_{16}$-inch neoprene.

Shaker. A Bodine motor, Type NC-1-12 RH, 1/50 hp., with a 30 to 1 reduction gear, furnishes power at 57 r.p.m. which is transferred to a Fisher clamp by a wheel, connecting arm, and crank arm as shown in Figure 5. A slot in the crank arm permits changes in the amplitude of the rocking motion.

Temperature Control. A centrifugal pump circulates water at 2.3 liters per minute between the jacket of the syringe and a constant-temperature bath (0.1° C. control by mercury regulator), in which the reactor is immersed as far as the shaker clamp will allow.

PROCEDURE

A water slurry containing about 1.3 grams of fresh Raney nickel was added to 40 ml. of solvent in the reactor.

The sample cup, containing a weighed sample equivalent to 0.5 to 12.0 millimoles of hydrogen, was hung on the hook attached to the reactor inlet tube.

The gaskets were very lightly oiled with Texaco Thuban 250 gear oil and placed on the flange of the reactor, then the sample cup and inlet tube were lowered carefully into the neck of the reactor, and the flanges were bolted together. A 10-pound torque on the end of a 6-inch wrench was more than enough to tighten the bolts and prevent hydrogen leakage.

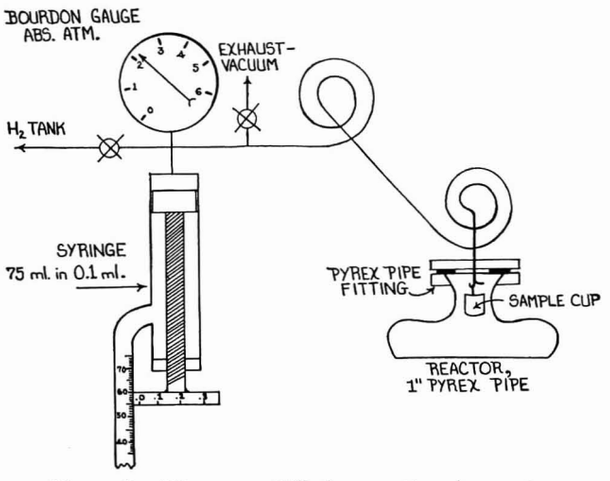


Figure 1. Diagram of Hydrogenation Apparatus

With the syringe set at a reading of 70 and the hydrogen valve closed, the apparatus was evacuated through the outlet valve, which was connected to the vacuum line. Then, without delay, the outlet valve was closed, and hydrogen was slowly admitted until the gage reading was 5 atmospheres absolute. This purging operation was repeated twice more to eliminate air from the system. On the last filling, the syringe screw was turned slowly to readings near 0.0 as hydrogen was admitted and the gage pressure was increasing. This avoided any possibility of drawing solvent vapors from the reactor into the syringe and gage.

The circulating pump was started, the bath temperature was adjusted, and the shaker was started. The hydrogen valve was

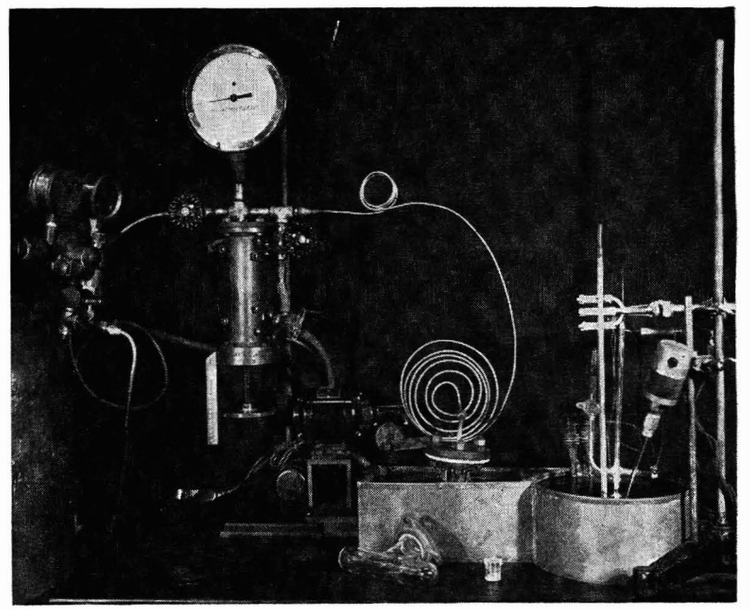


Figure 2. Component Parts of Apparatus

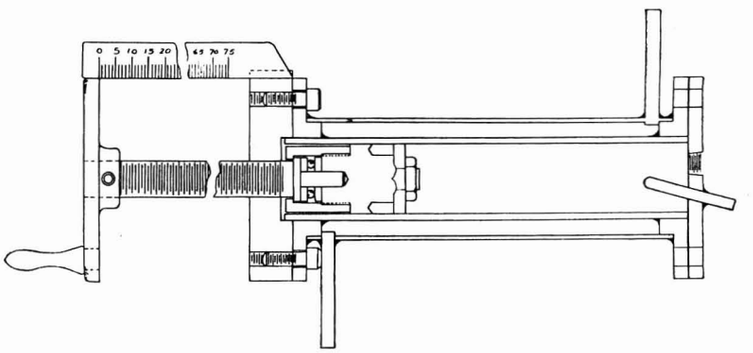


Figure 3. Syringe

opened as necessary to give a gage reading of 5.00 atmospheres (or any other desired working pressure above atmospheric) and then closed. Then the shaking was continued for 5 minutes while the catalyst and solvent were saturated with hydrogen; the pressure was maintained by adjusting the syringe. After this equilibration, the shaking was continued for 5 minutes more to let the operator look for leaks or changes in bath temperature.

Readings were made of bath temperature, and syringe reading was made at the exact desired working pressure. The shaker was stopped, and the reactor was unclamped and tipped so that the sample cup and its contents fell into the catalyst-solvent slurry. Then the reactor was clamped as before and shaking was resumed.

As hydrogen was used, the syringe screw was turned to maintain the original pressure. If necessary, the syringe could be refilled with hydrogen at a slight sacrifice in accuracy; during such refilling the shaker was stopped, so that hydrogen uptake was very slow.

Near the end of the hydrogenation, the reactor was unclamped and tipped so that solvent could wash down any sample particles adhering in the neck of the reactor. Then the shaking was resumed.

When the original pressure was maintained without further syringe adjustment, and the temperature was constant at its original reading, the syringe reading was recorded and used in the calculation

$$\text{Millimoles of } H_2 = \frac{P(V_2 - V_1)}{0.08206\ T}$$

where P is the gage reading in atmospheres, corrected for changes in barometric pressure, V_2 and V_1 are the final and initial syringe

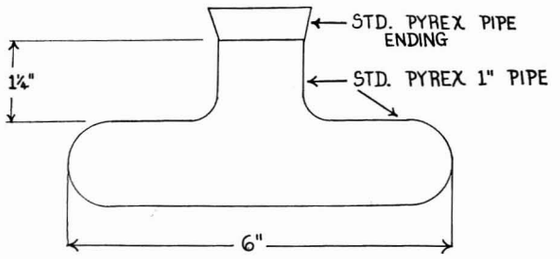


Figure 4. Reactor

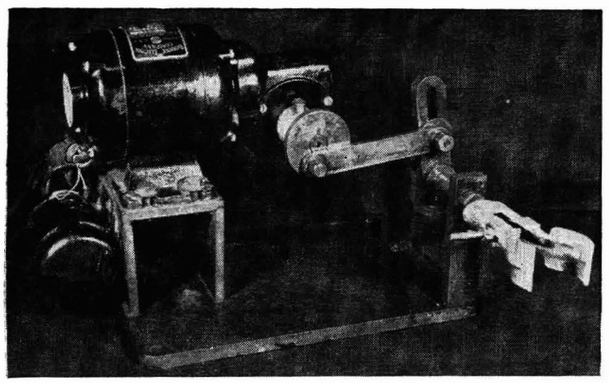


Figure 5. Shaker

readings in milliliters, and T is the absolute temperature of the bath.

RESULTS

Data from several typical hydrogenation experiments are given in Table I.

In these experiments 1.3 grams of Raney nickel and 40 ml. of solvent were used. The working pressure in all cases was 5.00 atmospheres, and the bath temperature was 25° to 30° C., except in the cases indicated, where temperatures near 0° C. were used.

Raney nickel was chosen for this work because of Whitmore and Revukas' demonstration (*5*) of its usefulness in azo splitting.

The cinnamic acid was J. T. Baker's purified U.S.P. grade recrystallized from methanol. It melted sharply at 134° C. corrected. The dye samples were purified until chromatographically homogeneous and recrystallized to constant melting point from methanol or ethanol.

The solvents were of reagent grade.

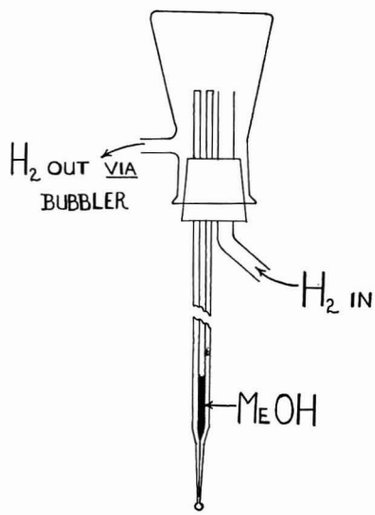
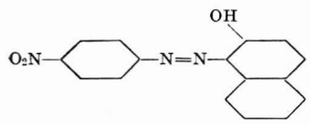
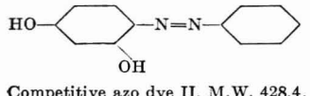
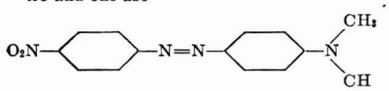


Figure 6. Arrangement for Diffusion Experiments

The hydrogen was purchased from the Air Reduction Co. (manufactured by the Paschall Oxygen Co., Philadelphia). It had a purity above 99.5%, as stated by the manufacturer and confirmed by the authors.

Table I. Tests of Hydrogenation Apparatus

Catalyst, 1.3 grams of fresh Raney nickel. Solvent, 40 ml. Pressure, 5.00 atmospheres. Temperature, room temperature or ice temperature

Test Substance	Wt., Grams	Solvent	Time, Min.	Millimoles H_2 Theory	Millimoles H_2 Found	% Error
Cinnamic acid	0.4947	EtOH	25[a]	3.34	3.39	+1.5
	0.9942	EtOH	20	6.71	6.69	−0.3
	1.0788	EtOH	30	7.28	7.30	+0.3
	1.0426	BuOH	20	7.04	7.09	+0.7
	0.9964	MeOH	30	6.72	6.71	−0.15
	1.0002	DMF[b]	20	6.75	6.76	+0.15
2,4-Dinitrodiphenylamine	0.3091	EtOH	105[a]	7.15	7.04	−1.5
	0.2612	EtOH	66	6.05	5.97	−1.3
	0.3941	EtOH	45	9.12	9.05	−0.8
	0.3160	EtOH	40	7.31	7.30	−0.1
	0.3307	BuOH	60	7.65	7.64	−0.1
	0.3505	BuOH	60	8.11	8.12	+0.1
Competitive azo dye I, M.W. 300.3, one nitro and one azo	0.4172	EtOH	90[a]	6.95	6.92	−0.4
	0.4283	EtOH	168[a]	7.13	7.12	−0.1
	0.3906	EtOH	20	6.50	6.45	−0.8
	0.3355	BuOH	30	5.59	5.53	−1.1
	0.2983	EtOH	180	5.80	5.82	+0.3
	0.9953	DMF[b]	35	9.29	9.23	−0.6
Competitive azo dye II, M.W. 428.4, one nitro and one azo	2.0003	EtOH	90	23.35 (Syringe refilled)	22.85	−2.1
	0.2024	DMF[b]	60	3.74	3.72	−0.5

[a] Ice temperature.
[b] Dimethyl formamide.

DISCUSSION

From the results in Table I it can be calculated that standard deviation of the errors is 0.7, excluding the next to the last case where the syringe was refilled. There is no correlation between the errors and either the temperature or the solvent employed. The error in the case of cinnamic acid, for example, is no worse with methanol as the solvent than with *n*-butyl alcohol. If a correction for solvent vapor pressure were required, the errors caused by neglecting it would be −3.2 and −0.17% for methanol and butanol, respectively (for reductions at 25° C. and 5 atmospheres).

The explanation for this lack of dependence on vapor pressure is believed to be that the 10-foot connecting tube between manifold and reactor serves as an effective barrier against diffusion of solvent vapor back into the syringe-gage system, particularly during a hydrogenation when there is a counterflow of gas to the reactor.

In order to estimate the diffusion rate of solvent through the connecting tube, the following experiment was carried out.

One end of a 2-foot length of borosilicate glass capillary, 1.25 mm. in bore, was drawn out to a fine tip. The other end was fastened through a 50-ml. filter flask as shown in Figure 6. Hydrogen gas was passed through the apparatus and forced through the capillary. The fine tip was dipped in methanol and a column of solvent allowed to rise in the capillary until the meniscus was 20 mm. above the drawn-down section; then the tip was removed and quickly sealed in the Bunsen flame. In this way, the solvent was sealed in the capillary under hydrogen.

Finally, the capillary was mounted in a vertical position, and hydrogen was passed slowly (0.5 ml. per minute) through the filter flask for several days. The average rate at which the meniscus receded was 2.5 mm. per day at 25° C.; this rate was not changed measurably during an additional 24 hours with an increased hydrogen flow of 15 ml. per minute.

In a control experiment, the capillary was evacuated, whereupon the methanol meniscus receded at a rate of 3 mm. per minute. The slow diffusion of methanol through hydrogen was thereby shown not to be a result of limitation by the extent of evaporation surface.

These results suggest that in this case correction for solvent vapor pressure is not justified because no significant amount of vapor can enter the measuring system under the specified operating conditions.

In the literature on laboratory hydrogenation at constant pressure, most experimenters who made a correction for solvent vapor had previously saturated their hydrogen before introducing it into their apparatus.

The closest analogy to the authors' apparatus is described by Jackson (*1*), who used a flexible glass helix and other connections totaling 3.4 meters of tubing 5 mm. in inside diameter (estimated) between measuring system and reactor. He did not saturate his hydrogen with solvent, but did make a correction for its vapor pressure. This correction may be justified because solvent vapor could reach his measuring system through a smaller diffusion barrier than the authors', at 1 atmosphere hydrogen pressure, during the 3 to 4 hours that Jackson allowed for temperature equilibration.

ACKNOWLEDGMENT

The authors are grateful to Lawrence T. Hallett for his interest and encouragement in this work, and to J. P. G. Beiswanger, D. L. Fuller, and Clyde McKinley for many helpful conversations on the diffusion effects discussed above.

To F. C. Snowden, and the draftsmen and machinists of this laboratory, the authors express their thanks for help in the design and construction of the apparatus.

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Organic Chemical Compounds in Raw and Filtered Surface Waters

HARRY BRAUS, F. M. MIDDLETON, AND GRAHAM WALTON
U. S. Public Health Service, Cincinnati 2, Ohio

KNOWLEDGE of the kinds and concentrations of organic chemical compounds in surface waters is important in studies concerned with tastes and odors in drinking water, natural purification of streams, analysis and tracing of industrial wastes, and toxic and other physiological effects on man and animal.

Direct determination of most organic compounds is not usually possible because of the minute concentrations that normally occur in surface waters. This study describes a method for the concentration and estimation of organic compounds in raw and filtered surface water. Possible applications of the techniques presented are discussed.

CONCENTRATION OF ORGANIC COMPOUNDS FROM RAW AND FILTERED SURFACE WATERS

The concentration of the organic compounds in the waters tested is accomplished by passing from 5000 to 75,000 gallons of water through small portable activated carbon filters. The average rate of flow through the filter is approximately 0.1 to 0.6 gallon per minute. Figure 1 shows the simplest type of activated carbon filter for use on filtered or finished water. This unit consists of a 3-foot length of iron pipe 4 inches in diameter. Between 1200 and 1500 grams of granular activated carbon are charged into this cylinder. The carbon is held in place by perforated cadmium-plated disks, equivalent to about a 16-mesh screen, soldered into the reducers at each end.

Figure 2 shows the assembly used when turbid or raw waters are sampled. The carbon filter is the same as that shown in Figure 1. It is preceded by an iron cylinder approximately half-full of filter sand and fine gravel. Valves are arranged so that the sand filter may be by-passed, back-washed with the raw water, or rinsed by filtering to waste. A minimum head of 10 feet of water is necessary for the effective operation of this unit.

A plastic filter, used for laboratory studies, consists of a 4-foot

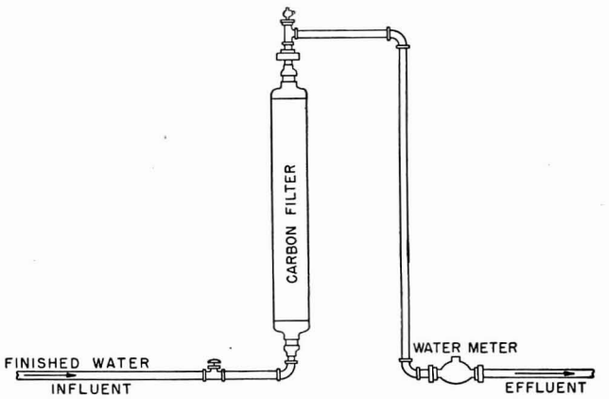

Figure 1. Carbon Filter Installation
Used for sampling filtered water for trace quantities of organic chemicals

This study was undertaken to develop a method for the recovery and identification of minute quantities of organic chemicals in drinking water and drinking water sources. Significant quantities of organic chemical compounds were recovered by passing water through activated carbon filters, followed by drying of the carbon and elution with an organic solvent. The organic residue was separated into five groups and certain specific chemicals were identified. Threshold odor tests were run on selected samples. For the first time, the effects of minute quantities of organic chemicals in water supplies can be studied in relation to: (1) the causes and treatment of objectionable tastes and odors in water, (2) industrial waste contamination in streams, (3) pollution caused by the growth and decay of aquatic plants and animals, and (4) possible biological or toxic effects on man or animals. The technique may be applied directly to industrial wastes of certain types and to other materials in dilute solution.

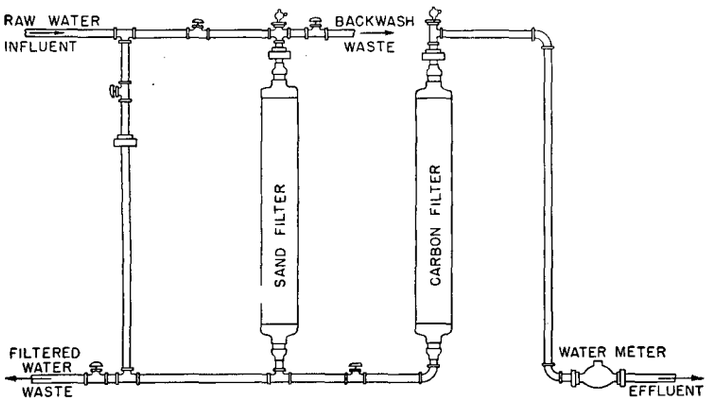

Figure 2. Sand and Carbon Filter Installation
Used for sampling turbid water for trace quantities of organic chemicals

length of plastic tubing, 3 inches in diameter. Carbon is held in place by a 60-mesh bronze screen at each end.

SELECTION OF CARBONS

Various types of activated carbon were tested for use in these experiments. Cliffchar (4- to 10-mesh) granular carbon was used in the filters placed in the field. A more active carbon of finer mesh would have been desirable for these studies, but to maintain satisfactory flows the coarse carbon was used. Studies of finished water under laboratory controlled conditions were made with unground C-190 activated carbon, furnished by the Industrial Chemical Sales Division of West Virginia Pulp and Paper Co.

Table I. Ether-Extractable Materials in Two Activated Carbons

Carbon	Ether-Extractable Materials, %
Cliffchar, granular	0.0117
Nuchar C-190, unground	0.0027

Two laboratory filters, 15 × 3 inches, were set up in series, and 12,000 gallons of Cincinnati drinking water were passed through them at a rate of 0.2 gallon per minute. A total of 3.25 grams or 70 parts per billion of material were extracted from the first filter in the series. The second filter, which received the effluent from the first filter yielded 1.26 grams of extract or 28 parts per billion.

These carbons contain some ether-extractable materials as received from the manufacturer. Results of ether-extraction tests made with carefully purified ether are shown in Table I.

Nuchar C-190 carbon has less than one fourth as much ether extractable material as does the Cliffchar carbon. It is not believed that the extracted materials interfere with the test made on the total organic residue. A comparison of the adsorptive ability of these carbons was made using Cincinnati tap water. Approximately 8000 gallons of water were passed through each of two filters containing the carbons during the same period (Table II). Approximately five times as much material was recovered from the same quantity of water using the C-190 carbon.

STUDIES ON FIELD FILTERS

Filters of the type described were placed in water plants at various cities on the Ohio River and at other locations having surface water supplies. An effort was made to include certain locations known to be subject to industrial waste pollution and others known to be free of such pollution. Results of these studies are discussed below.

Table II. Organic Material Extracted from Equal Volumes of Same Cincinnati Tap Water by Two Types of Activated Carbon

Type of Carbon	Weight of Carbon Used	Total Organic Material Extracted[a]	
	Grams	*Grams*	*P.p.b.*[b]
Nuchar C-190	902	4.016	133
Cliffchar	1900	0.81	27

[a] Net weight after correction for organic extractable material from carbon.
[b] Parts per billion.

ANALYTICAL PROCEDURES

After a quantity of water has been passed through the filters, they are returned to the laboratory and dismantled. The carbon is removed, air-dried on a 60-mesh bronze screen, then placed in a large-capacity modified Soxhlet extractor (Corning No. 3885, Catalog LP28), and extracted with diethyl ether. A good grade of ether is selected. Care must be used to keep at a minimum the formation of peroxides in the ether. The U.S.P. test for peroxides in ether should be applied. Purification of ether may be accomplished as follows: The ether is shaken with acidulated ferrous sulfate, washed with distilled water, and dried with anhydrous calcium chloride. After standing several days over the calcium chloride, the ether is filtered through Whatman No. 12 filter paper and distilled.

Great care must be exercised in starting the extraction, because a large amount of heat of adsorption is evolved. The authors have found it reasonably safe to add carbon slowly to the cold ether previously placed in the Soxhlet extractor.

After extraction, the ether extract is evaporated to a volume of 25 ml. Anhydrous sodium sulfate is added to the ether solution and the whole is allowed to stand 48 hours. The ether

Table III. Total Anhydrous Organic Extract in Various Surface Water Supplies

Location of Water Plant	Filter in Service From	Filter in Service To	Sampling Point	Quantity of Water[a] Gal.	Organic Material Extracted Grams	Organic Material Extracted P.p.b.[b]
Midland, Pa.	2/14	7/14	Filter effluent	19,275	1.275	17.5
Wheeling, W. Va.	2/2	8/9	Filter effluent	20,560	1.575	21.0
Huntington, W. Va.	5/28	7/25	Finished water	23,550	1.795	21.0
Pomeroy, Ohio	1/24	5/11	Filter effluent	40,000	1.420	9.3
Cincinnati, Ohio	6/1	8/1	Raw water	4,000	0.485	32.0
Louisville, Ky.	4/14	8/3	Filter effluent	28,000	1.160	10.7
Columbus, Ohio	5/28	9/15	Filter effluent	80,800	4.000	13.0

[a] Metered flow of water through Cliffchar carbon.
[b] Parts per billion.

solution is then filtered and the residue is washed with four 5-ml. portions of anhydrous ether. The residue is discarded. The ether solutions are combined in a tared flask and gently evaporated in the hood. The flask and contents are dried in a vacuum desiccator over calcium chloride and weighed. The net weight represents the total ether-extractable material found on the carbon.

The ether-extractable material is now subjected to group separation, which is effected according to a modification of the method of Shriner and Fuson (*3*).

The dry ether-soluble residue is taken up in 25 ml. of ether, placed in a separatory funnel, and extracted using four 10-ml. portions of 5% hydrochloric acid in which is dissolved 20% sodium chloride. The hydrochloric acid–sodium chloride extracts are combined and rendered alkaline with 5% sodium hydroxide solution. The resulting mixture is extracted with four 10-ml. portions of ether. The ether layers are combined and dried over anhydrous sodium sulfate. The dried ether solution is filtered to remove the sodium sulfate. The ether is evaporated and the residue is weighed after desiccation over calcium chloride. The residue is composed of organic basic compounds, largely amines.

The basic water solution now contains the amphoteric compounds. It is carefully neutralized with acetic acid and extracted with five 10-ml. portions of ether. The ether extracts are combined and dried as previously. The ether is evaporated and the residue is desiccated over calcium chloride and weighed. This residue is composed of amphoteric compounds.

The ether layer remaining from the hydrochloric acid extraction is now extracted with four 10-ml. portions of 5% sodium hydroxide containing 20% sodium chloride. A soapy emulsion formed at this point may be broken by the addition of a drop of ethyl alcohol or a little more water. The ether layer is dried with anhydrous sodium sulfate, filtered, and evaporated. The residue remaining is weighed as a mixture of neutral organic compounds.

The neutral residue so obtained is usually a complex mixture. No general method of analysis is available, but certain methods achieve remarkable separation in many instances. The methods commonly resorted to are fractional crystallization, distillation, and sublimation. When separation is completed, the individual components may be further classified.

The sodium hydroxide–sodium chloride solutions are combined, cooled, and saturated with gaseous carbon dioxide. The carbon dioxide–saturated solution is now extracted five times with 5-ml. portions of ether, dried, and evaporated. The residue is weighed after drying and is composed largely of phenolic compounds.

The sodium bicarbonate–sodium chloride solution is now gently heated and stirred to remove any dissolved ether. It is then cooled, made acid to litmus with dilute hydrochloric acid, and extracted with four 10-ml. portions of ether. After drying and evaporation of the ether, the residue is weighed. This residue is composed of strong acids and highly acidic phenols.

Throughout the whole procedure semimicro or micro equipment is used wherever possible. Sodium chloride is added to all aqueous extractants to decrease the solubility of the ether and generally to render more quantitative separations. All volumes are kept as low as possible. Because of the small amounts of materials involved, thorough washing and shaking in extractions are necessary.

When separation of the groups is completed, a micro qualita-

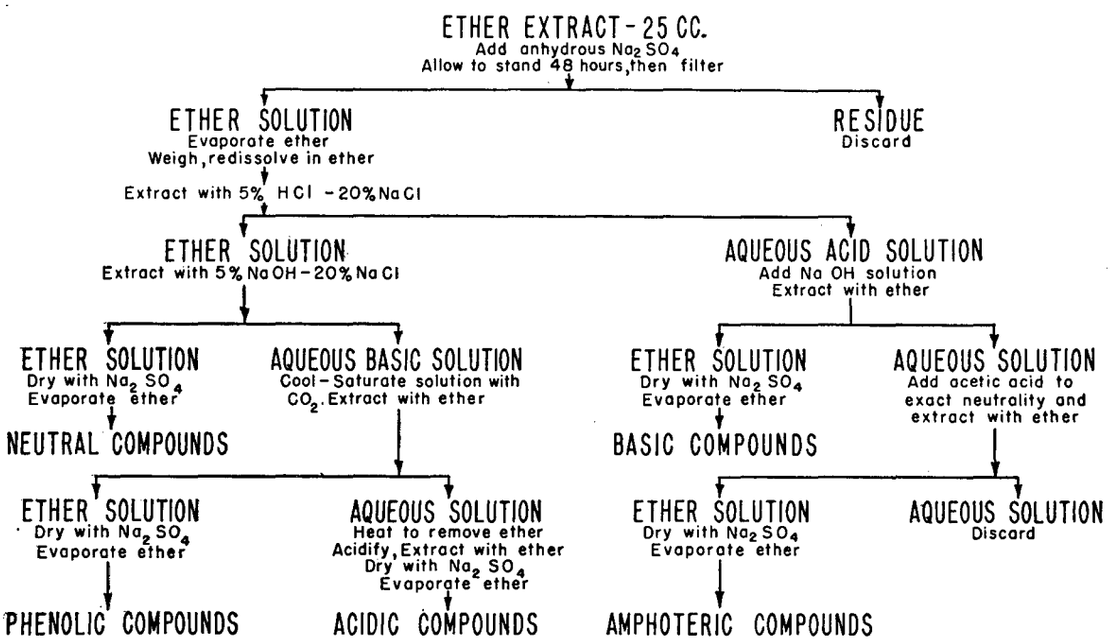

Figure 3. Separation of Mixture of Organic Compounds Modified According to the Method of Shriner and Fuson

tive test for the elements nitrogen, sulfur, and chlorine is made. Nitrogen and sulfur are determined by the method of Feigl (*1*).

RESULTS

Carbon (Cliffchar) filters were placed in service at six water plants along the Ohio River from Midland, Pa., to Louisville, Ky., during the spring of 1949. An additional carbon filter was placed in service at Columbus, Ohio (Scioto River). After a metered volume of water had passed through the carbon filters, the filters were returned to the laboratory and the carbon was subjected to the treatment described above.

Pertinent data relative to the sampling locations, period of sampling, quantity of water passed through the filters, and the total quantity of organic matter extracted from the carbons are presented in Table III. The total amount of organic material extracted, as shown in the last column of Table III, varied from 9.3 to 32 parts per billion. These values may be subject to significant error due to variable filtration rates and metering errors. The extract from the Columbus, Ohio, sample contained appreciable quantities of organic compounds. This supply is relatively free from industrial pollution.

Cincinnati tap water was passed through a filter containing Nuchar C-190 carbon. The ether extract from the carbon was deep yellow in color. On evaporation a black viscous residue of foul odor was obtained. Elemental analysis of this residue indicated the presence of nitrogen, sulfur, and halogen. The residue was then subjected to qualitative group separation according to the scheme shown in Figure 3.

The hydrochloric acid–sodium chloride extraction was continued until most of the yellow color was extracted. The sodium hydroxide–sodium chloride–water extraction yielded three layers. The top ether layer was colored amber, while the lower aqueous layer changed to an orange color indicative of phenolic materials. Between these two layers a dark oily middle layer of a tarry consistency was noted. The three layers were separated and the tarry mixture was recovered.

The middle layer was found to be soluble in very dilute sodium hydroxide but insoluble in 5% or stronger sodium hydroxide as well as in water, hydrochloric acid solution, and petroleum ether. It was soluble in ethyl alcohol, benzene, carbon disulfide, and ether. Crystallization could not be induced from any of these solvents or mixtures thereof. The compound was purified by dissolving in very dilute sodium hydroxide and reprecipitating with dilute hydrochloric acid. After several such reprecipitations a light brown amorphous powder was obtained.

Table IV. Lowest Concentration of Organic Extract Detectable by Water-Dilution Method of Odor Testing

Source of Water[a]	Lowest Concentration Detected by Odor Test
	P.p.b.
Huntington, W. Va.	13
Wheeling, W. Va.	25
Louisville, Ky.	13
Columbus, Ohio	3
Pomeroy, Ohio	6
Cincinnati, Ohio	25
Organic groups	
Phenolic	50
Neutral	11
Organic acids	160

[a] All sources of water were filter effluents before chlorination, except at Cincinnati, where water treatment includes breakpoint chlorination.

It was thought that this substance might be a complex phenol and that the anomalous behavior was caused by the sodium salt. Subsequent tests, however, did not indicate the compound to be of phenolic nature. The compound could not be acetylated, and on reaction with Gibbs reagent gave a negative response. Using the Liebermann-Storch reaction a positive violet color was obtained, indicating a rosin-type compound. A terpenelike odor was noted.

A positive test for chlorine was obtained in the phenolic group. Reaction with the Gibbs reagent gave a green color indicative of chlorinated phenols. Separation of the individual phenols was not attempted. The phenolic groups as separated had the typical phenol odor.

The acid groups were characterized by an odor similar to caproic acid. The group was isolated as a viscous, almost solid mass of brown color. Microdistillation yielded a residue of very high boiling point, which had the general properties attributed to fatty acids of high molecular weight. It is conjectured that these acids may have originated in the large amount of laundry wastes dumped into the river. The basic group had what is best described as a tobaccolike odor. Pyridine or pyridine-type compounds were shown to be present, using the micro test of Feigl (*2*).

The organic neutral compounds appear to be the largest group of compounds isolated. This group is characterized by a particularly offensive odor. No identifications of the constituents of this group have as yet been attempted. However, the pine splinter–hydrochloric acid test was positive on the ether extract. The red color formed is indicative of pyrrole or pyrrole derivatives. Their presence in the neutral groups would in many cases be consistent with solubility considerations.

In the analysis of a different filter which was in operation along the Ohio River below a refinery, relatively large amounts of hydrocarbons were isolated from the neutral group. It was subjected to micro steam distillation and the distillate was extracted with ether. Upon evaporation of the ether and solution of the residue in fuming sulfuric acid, a floating layer resulted which is highly indicative of aliphatic hydrocarbons.

The amphoteric group is characterized by little or no odor. This is consistent with the presence of more than one polar group. No separation or identification of this group has been attempted.

POSSIBLE APPLICATIONS OF TECHNIQUE

Taste and Odor Studies. Tastes and odors in water supplies may arise from industrial and domestic wastes or from natural causes, such as the growth and decay of aquatic plants and animals. In any case, most of the compounds causing objectionable tastes and odors are organic in nature.

The concentration, extraction, and separation procedures presented in this paper provide a method for obtaining sufficient quantities of the organic compounds for study. By actual taste and odor tests the organic groups or individual compounds most likely to cause tastes and odors may be determined. The effect of these organic compounds may be studied on water-treatment processes, natural purification in streams, and removal from water or wastes.

Threshold concentrations of taste and odor were determined on the total organic extract of certain samples and on the various organic groups separated (Table IV). Examination of Table IV reveals that 3 to 25 p.p.b. of the total organic extracts from the various waters gave detectable odors when tested in the laboratory by the water dilution method. No attempt has been made in this study to compare the threshold odor concentrations of the various organic extracts with the actual odors occurring in the water supplies from which the extracts were obtained. Carbon filters were purposely set up to receive the rapid sand filter effluents at the water plants, to avoid the possible effects of chlorination on the taste- and odor-producing substances.

The extract from the Columbus, Ohio, water supply, which is not subject to industrial pollution, produced detectable odor in a concentration of 3 p.p.b. The extract obtained from Cincinnati tap water produced detectable odor at a concentration of 25 p.p.b. This water had undergone complete treatment, including breakpoint chlorination.

The phenolic, neutral, and organic acid groups which were separated from the Cincinnati tap water extract were tested for

odor-producing characteristics. The neutral group was detected at a concentration of 11 p.p.b., and the phenolic and acid groups at a concentration of 50 and 160 p.p.b., respectively.

Tracing Industrial Wastes. Isolation of the organic compounds gives a clue as to the type of industry responsible for contributing the waste material. By this means a contributor of a given waste may be identified. The method has also proved useful in the direct examination of certain industrial wastes.

Study of Reactions in Dilute Solution. The concentration method is useful in the study of certain reactions in dilute solution. The reactions of chlorine and phenol in dilute solution are being studied in this laboratory. To obtain a sufficient amount of the reaction product for study, large quantities of the dilute solutions are treated with active carbon. The carbon is removed by filtration, followed by ether extraction and recovery of the product formed in the reaction. These chlorinated phenolic products have been isolated and their identification is being investigated. Other reactions that may be studied include the products formed by use of chlorine dioxide and ozone when added to various compounds present in water.

Possible Basis for Physiological Study. The physiological effect, if any, of small concentrations of organic compounds in drinking water has not been studied. The method presented is helpful in providing sufficient material for studies of a physiological nature.

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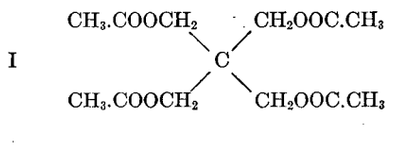
NOTES ON ANALYTICAL PROCEDURES . . .

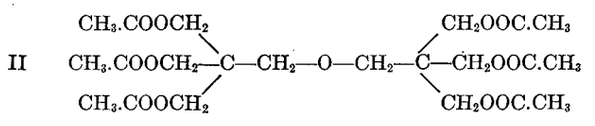
Determination of Dipentaerythritol in the Presence of Pentaerythritol

JOSEPH H. JAFFE AND SHRAGA PINCHAS
Weizmann Institute of Science, Rehovoth, Israel

FRIEDRICH and Brün (*2*) have shown that the pentaerythritol prepared by condensation of formaldehyde and acetaldehyde in the presence of calcium hydroxide is always accompanied by a certain and sometimes an appreciable proportion of dipentaerythritol which cannot be removed by any simple method. As for some purposes, only limited quantities of dipentaerythritol are permissible, a quick and easy method for the estimation of the latter in a mixture is important. The methods of Kraft (*3*) and Wyler (*6*), in which the pentaerythritol is determined quantitatively, can (*1*) be used for the estimation of dipentaerythritol only if one is sure that no other impurities are present in the sample of pentaerythritol, while the chromatographic method suggested by Lew and coworkers (*4*) is somewhat lengthy.

An attempt was therefore made to estimate dipentaerythritol in the presence of pentaerythritol by infrared spectroscopy. As both substances dissolve in nonpolar solvents with great difficulty and polar solvents are generally unsuitable for this purpose, the absorption of the corresponding acetates—namely, pentaerythritol tetraacetate (I) and dipentaerythritol hexaacetate (II)—was measured in carbon tetrachloride solution.





The only significant differences between the spectra of these compounds were to be expected in the regions of the absorption of the $H_2C—O—CH_2$ linkage, which occurs in II only. An absorption band due to this group has been reported (*5*) at about 1120 cm.$^{-1}$ This band was found for II at 1115 cm.$^{-1}$ (Figure 1); a neighboring band of the carbon tetrachloride may somewhat distort it at very low concentrations of II. At this frequency, I has a weak general absorption, but there is no trace of a band. Synthetic mixtures of I and II were therefore prepared, and it was shown that even in the presence of much I the optical densities at 1115 cm.$^{-1}$ permitted a reasonably good estimation of II in the mixture.

It is suggested that dipentaerythritol be determined in crude pentaerythritol by acetylation (*1*) and determination of the optical density of a dilute solution of the total resulting acetates in carbon tetrachloride at 1115 cm.$^{-1}$

The range investigated is 10 to 70% dipentaerythritol in pentaerythritol. For quantities of the contaminant much smaller than 10%, it will probably be advisable to enrich the dipentaerythritol in the sample before applying the method of assay described. Any higher "polymer" of pentaerythritol will be determined by this method as dipentaerythritol.

EXPERIMENTAL

The infrared absorption measurements were made with a Perkin-Elmer, Model 12 C spectrometer, using a rock salt prism and a slit width of about 0.4 mm. In order to minimize interaction between I and II, very dilute solutions (total concentration of about 0.01 gram per ml.) were used and a cell of 2-mm. thickness was constructed so as to obtain a sufficient absorption. The cell consisted of a pair of rock salt plates, between which a lead ring was amalgamated as spacer. Samples were introduced by means of a hypodermic syringe needle, through two small holes drilled in the spacer. During the measurements, these holes were closed with pins in order to reduce evaporation of the solvent. In the quantitative work, it was found more accurate to set the spectrometer at 1115 cm.$^{-1}$, the absorption maximum of the $H_2C—O—CH_2$ group, than to measure the complete absorption spectrum. A blank measurement with the pure solvent was carried out immediately before that with the solution, and the difference was taken as the optical density of the solution.

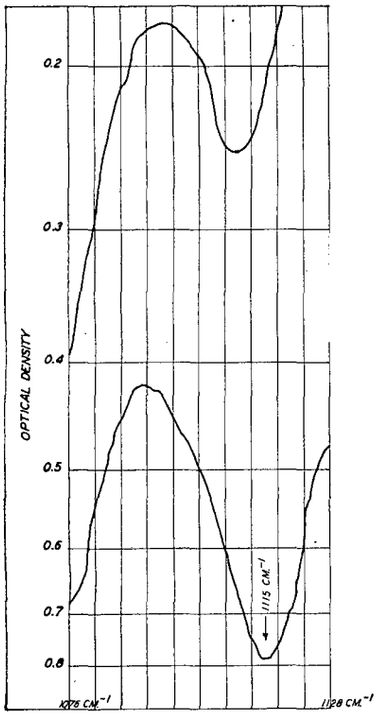


Figure 1. Infrared Spectra

Upper. **Carbon tetrachloride**
Lower. **Dipentaerythritol hexaacetate (0.007 gram) in 1 ml. of carbon tetrachloride**
2-mm. cell

Experiments using acetonitrile as solvent gave less satisfactory results than those obtained with carbon tetrachloride (Tables I and II). The propionates in a variety of solvents gave inferior results to the acetates.

Expt. No.	Pentaerythritol, G.
1	4.0
2	1.0
3	1.0
4	2.0
5	1.0

The pure compounds, I and II, were prepared from pure pentaerythritol and dipentaerythritol, respectively, by the method of Bograchov (*1*). The polyvalent alcohols (10 grams) were refluxed for 6 hours with acetic anhydride (50 grams) and anhydrous sodium acetate (15 grams). The hot mixture was poured into an excess of water (100 ml.), and the solid was filtered, dried, and recrystallized repeatedly from petroleum ether. Melting point of I, 85–87° C.; of II, 73° C.

Table I. Results on Pure Compounds

Concentration, G./Ml.	Optical Density	Extinction Coefficient, for 1 G./Ml., 1-Mm. Cell
0.00396 (II)	0.325	41
0.00368 (II)	0.30	41
0.0035 (II)	0.29	41.5
0.0069 (I)	0.085	6
0.018 (I)	0.21	6
0.025 (I)	0.27	5.5
0.0405 (I)	0.49	6

For I, very nearly the same extinction coefficient was found for solutions in acetonitrile.

In calculating the percentage of II in the mixtures it is assumed that no other compound was present and the absorption of I at 1115 cm.$^{-1}$ is taken into account. The following equation therefore obtains, according to Beer's law:

$$d = cl\left[41\frac{x}{100} + 6\left(1 - \frac{x}{100}\right)\right]$$

Table II. Results on Mixtures of Pure Compounds

Concentration, G./Ml. II	Concentration, G./Ml. I	Optical Density, d (Obsd.)	% of II Calcd. from d	% of II Actual	Deviation, %
0.00128	0.0116	0.253	10.9	9.9	+1.0
0.0021	0.0158	0.368	12.3	11.7	+0.6
0.00234	0.0142	0.37	15.0	14.2	+0.8
0.00256	0.0061	0.285	30.0	29.6	+0.4
0.00704	0.0086	0.68	45.2	45.0	+0.2
0.00604	0.00238	0.515	70.4	71.7	−1.3

where d = optical density measured, c = total concentration in grams per ml., l = cell thickness in mm., and x = percentage of II in the mixture. Hence,

$$x = \frac{d \times 100}{c \times l \times 35} - 17.1 = \frac{2.86 \times d}{c \times l} - 17.1$$

Analytical Procedure. Mixtures of accurately weighed quantities of pentaerythritol and dipentaerythritol were refluxed for 6 hours with five times their weight of acetic anhydride and 1.5 times their weight of anhydrous sodium acetate. The reaction mixture was cautiously diluted with water (ten times the weight of the initial mixture) and, after the excess anhydride had been hydrolyzed, extracted repeatedly with carbon tetrachloride. The solution was filtered through a dry fluted filter and the final volume noted.

For the absorption measurements in the infrared, greatest accuracy is achieved when a dilution is used which gives an optical density of the order of 0.2 to 0.5.

Dipentaerythritol, G.	Final Volume, Ml.	Optical Density, d	Dipentaerythritol, % Calcd.	Found	Deviation
0.5	400	0.493	12.4	11.1	+1.3
1.0	812.5	0.230	48.5	50.0	−1.5
2.0	900	0.393	66.8	66.7	+0.1
0.5	450	0.305	20.6	20.0	+0.6
0.5	225	0.490	34.3	33.3	+1.0

To the calculation of the percentage, x, of dipentaerythritol in the mixture, the following formulas apply:

$$d = \frac{a \times \frac{x}{100} \times 1.99}{v} \times l \times 41 + \frac{a\left(1 - \frac{x}{100}\right) \times 2.24}{v} \times l \times 6$$

$$x = \frac{1.47 \times d \times v}{a \times l} - 19.7$$

where

a = weight of sample, grams
v = final volume of solution in carbon tetrachloride, ml.
l = length of cell, mm.
d = optical density of solution at 1115 cm.$^{-1}$
1.99 = ratio of molecular weights of (II) (506) and dipentaerythritol (254)
2.24 = ratio of molecular weights of (I) (304) and pentaerythritol (136)

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RECEIVED December 12, 1949. Investigation carried out under the auspices of the Scientific Department, Israeli Ministry of Defence, and published with its approval.

Sample Temperature in the Carrier-Distillation Arc

SOL WEXLER[1]

Los Alamos Scientific Laboratory, Los Alamos, N. M.

SCRIBNER and Mullin (*2*) have described a carrier-distillation procedure for the determination of trace impurities in uranium, in which 2% gallium oxide is added to the uranium oxide sample and the intimate mixture is excited in the anode of a direct arc. The gallium oxide "carrier" is stated to stabilize the arc and to sweep out the minute amounts of impurities volatilized by heat from the electrode, thus effecting a separation from the more refractory uranium oxide. The degree of volatilization of each impurity oxide should depend on its vapor pressure at the charge temperature during the course of arcing. With the usual current of 10 amperes, distillation of even the more volatile oxides into the arc is not complete in one or in some cases several burnings. Impurity spectral lines are observed when the spent uranium oxide charges are mixed with fresh carrier (2 mg. of gallium oxide) and re-arced (*1, 2*).

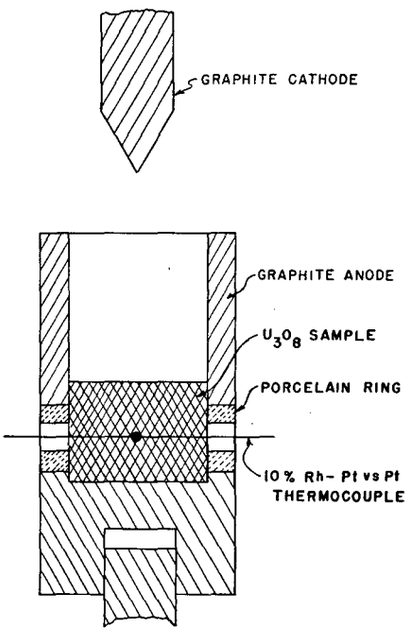

Figure 1. Hot-Junction Thermocouple Assembly

A knowledge of the sample temperature would be helpful in understanding the incomplete volatilization of impurities as well as other questions relating to the mechanism of the carrier-distillation method. Scribner and Mullin have estimated the charge temperature to be near 2000° C. when the current is 10 amperes. This value is based on their observation that the charge sinters to a fine cake but does not melt on arcing, which may indicate a temperature slightly below the melting point of uranium oxide. In addition, they measured the temperature of the outer surface of a sample-bearing electrode with an optical pyrometer and found it to vary from 2300° C. near the bottom to 2800° C. near the top. In this paper are described two methods of determining the temperature of 100-mg. uranium oxide synthetic samples, with and without carrier. Sample temperatures during the course of arcing are measured with a thermocouple embedded in the powdered uranium oxide. These are compared with optical observations made on samples through holes in the sides of the burning craters.

[1] Present address, Argonne National Laboratory, Chicago, Ill.

PROCEDURE

The hot junction of the 10% rhodium–platinum alloy–platinum thermocouple (Figure 1) was embedded in synthetic 100-mg. uranium oxide charges (containing about 30 elements in 10 p.p.m. concentrations). Each 20-mil wire was led through a small hole in the electrode wall and shielded from the graphite by a porcelain collar. The sample holder was the usual 0.25-inch (0.6-cm.) spectroscopically pure graphite with a crater 4 mm. across and 8 mm. deep. Opposite was a pointed 0.125-inch graphite rod, with an electrode gap of 4 mm. A Dietert-Applied Research Laboratory direct current supply energized the arc. Readings of electromotive force were taken with a Leeds & Northrup potentiometer indicator. Because the sample temperature increased very rapidly in the first several seconds of burning, it was found advisable to set the indicator at progressively increasing values and then record the times when the galvanometer needle swept past zero.

Temperature measurements were also taken with a Leeds & Northrup optical pyrometer focused on a side of the sample through a 1.5-mm. hole in the crater wall.

RESULTS AND DISCUSSION

The sample temperature of 2% gallium oxide in uranium oxide mixtures exhibits a very rapid initial rise, followed by a leveling off (Figure 2). As one would expect, the rate of increase and the magnitude of the steady-state temperature vary directly with the arc current. The establishment of a fairly constant sample temperature speaks for a stable though steep temperature gradient down the electrode.

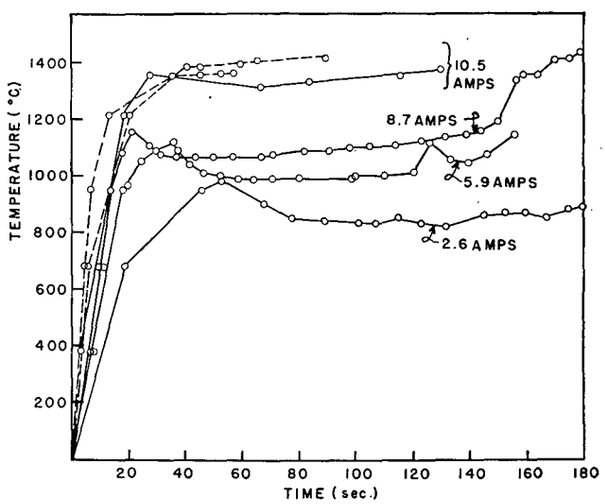

Figure 2. Temperature Rise with Time as Function of Arc Current

2% Ga_2O_3

The carrier substance was changed and the current kept constant at 10.5 amperes in a second set of experiments (Figure 3), in which 2% gallium oxide (boiling point about 1800° C.), 4% silver chloride (1564°), 5% barium fluoride (2257°), and 6% cadmium oxide (1385°) were selected to give a reasonably wide range of carrier volatility. The concentration of the carrier was that necessary to give the same length of silent period for each charge. Included are two runs with uranium oxide to which no carrier was added. Evidently, the temperature during

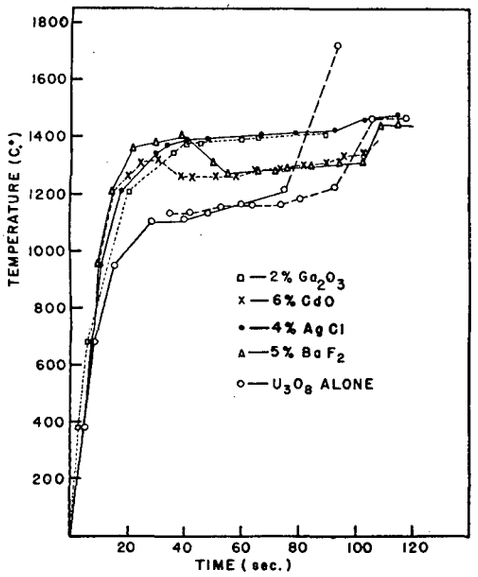

Figure 3. Temperature-Time Relations for Various Carriers

Current, 10.5 amperes

the usual exposure time (the first 35 to 45 seconds) is little dependent on the particular carrier substance. However, the presence of the carrier appears to raise the sample temperature some 200° to 300° over the approximately 1100° C. observed for uranium oxide alone. This suggests that the increased analytical sensitivity due to addition of carrier is in part the result of the higher sample temperature.

Steady-state temperatures, with a current of 10.5 amperes, obtained earlier from the pyrometric method are assembled in Table I. The 1200° to 1500° C. temperature range is in good agreement with that from the thermocouple measurements. However, the pyrometer procedure is believed to be less accurate by reason of difficulty in focusing the instrument exactly on the hole, a precaution necessitated by the steep temperature gradient down the electrode.

Table I. Steady-State Temperatures Measured with Optical Pyrometer

Carrier (in 100 Mg. U_3O_8)	Temperature, °C.
2% Ga_2O_3	1435
	1595
	1320
	1450
2.5% BaF_2	1225
	1220
2% AgCl	1335
	1375
	1355
2% CdO	1275
	1340
	1220
U_3O_8 alone	1400

The thermocouple measurements indicate that the sample is at its maximum temperature for less than half the usual exposure period. Furthermore, the steady-state temperature appears to be lower than the boiling points of most of the impurity oxides in uranium oxide. These observations may explain in part the lack of complete volatilization of the impurities in a single arcing.

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Determination of Parathion and *p*-Nitrophenol in Technical Grade Materials and in Dust Preparations

KATHRYN O'KEEFFE AND P. R. AVERELL

Stamford Research Laboratories, American Cyanamid Co., Stamford, Conn.

THE widespread use of parathion (*O,O*-diethyl *O*-*p*-nitrophenyl thiophosphate) as an agricultural insecticide necessitated the development of procedures for the analysis of the technical product as well as dust and wettable powder preparations.

To date the only method reported for the analysis of technical parathion is the polarographic procedure of Bowen and Edwards (*3*), in which the nitro group is reduced at the dropping mercury electrode. For the estimation of small amounts of parathion, Averell and Norris (*2*) have described a colorimetric method involving the reduction of parathion to the amino derivative, which on diazotization and coupling with *N*-(1-naphthyl)-ethylenediamine dihydrochloride gives a dye; this procedure has been modified by Gage (*6*). Although this procedure has been used extensively for the estimation of residual parathion in plant and animal tissue, the accuracy to be expected from a colorimetric procedure makes it unsuitable for the analysis of technical parathion or concentrated dust preparations thereof.

This paper describes a method which has been used in the authors' laboratory for over 3 years for the assay of technical grade parathion and of dust formulations. Parathion is separated from *p*-nitrophenol, the most likely impurity, by mild alkaline extraction of an ether solution; the *p*-nitrophenol in the aqueous layer is determined colorimetrically as sodium *p*-nitrophenoxide; the parathion in the ether layer is reduced by zinc and hydrochloric acid; and the amino group so formed is titrated with standard sodium nitrite solution. This reduction-nitrite titration procedure is essentially that described by Callan and Henderson (*4*).

ANALYSIS OF TECHNICAL PARATHION

Reagents. Ethyl ether.

Sodium carbonate, 1%, 10 grams of sodium carbonate per liter of solution

Sodium hydroxide, 1 *N*, 40 grams of sodium hydroxide per liter of solution

Sodium hydroxide, 0.1 *N*, 1 volume of 1 *N* sodium hydroxide in 9 volumes of water

Acetic acid–hydrochloric acid, 9 volumes of glacial acetic acid in 1 volume of concentrated hydrochloric acid

Sodium nitrite solution, 0.1 *N*, 6.90 grams of sodium nitrite per liter of solution, standardized against anhydrous sulfanilic acid

Sodium (or potassium) bromide, C.P.

Zinc dust, iron-free

Hydrochloric acid, concentrated

Potassium iodide–starch paper

Sulfanilic acid, Eastman Kodak

Instrument. A photoelectric colorimeter equipped with a cell 1.5 cm. in diameter and a filter to give maximum transmittance between 400 and 450 mμ. A spectrophotometer may be used, although the filter photometer gives adequate precision.

Procedure. SEPARATION OF PARATHION AND *p*-NITROPHENOL. Weigh accurately from a weighing pipet 0.6 to 0.9 gram of sample into 100 ml. of ethyl ether contained in a 250-ml. separatory funnel. Extract the ether solution four times (or until the extract is colorless) with 20-ml. portions of chilled, 1% sodium carbonate solution, collecting the combined aqueous layers in a 200-ml. volumetric flask. Transfer the ether layer to a 400-ml. beaker, using small portions of ether to effect the quantitative transfer.

DETERMINATION OF *p*-NITROPHENOL IN AQUEOUS EXTRACT. Preparation of Standard Curve. Weigh accurately 100 mg. of *p*-nitrophenol, transfer to a 1-liter volumetric flask, and make up to volume with 0.1 *N* sodium hydroxide. Transfer 2-, 4-, 6-, 8-, 10-, and 20-ml. aliquots of this solution to 100-ml. volumetric flasks and make each solution up to volume with 0.1 *N* sodium hydroxide. Read the light transmittancy of each of the standard solutions at 400 mμ by means of a photoelectric colorimeter which has been set to give 100% transmittance with water. Plot the light transmittancies as abscissas against the concentrations (milligrams per milliliter) as ordinates on semilogarithmic graph paper.

Table I. Analysis of Purified Parathion

Parathion, Gram		Purity,
Added	Found	%
0.8167	0.8142	99.7
0.6110	0.6122	100.2
0.7868	0.7829	99.5
0.7350	0.7337	99.8
0.7196	0.7182	99.8
	Av.	99.8

Determination of *p*-Nitrophenol. Add 20 ml. of 1 *N* sodium hydroxide to the combined aqueous extracts contained in the 200-ml. volumetric flask and make up to volume with water. Measure the light transmittancy of the solution at 400 mμ with a photoelectric colorimeter and read from the standard curve the concentration of *p*-nitrophenol in milligrams per milliliter of solution.

$$\% \text{ } p\text{-nitrophenol} = \frac{\text{mg./ml.} \times 200 \times 100}{1000 \times \text{grams of sample}}$$

A dilution of the sodium *p*-nitrophenoxide solution to 200 ml. is suitable for technical parathion containing up to 0.2% *p*-nitrophenol. If the sample being analyzed contains greater amounts of *p*-nitrophenol, the sodium *p*-nitrophenoxide solution must be diluted with 0.1 *N* sodium hydroxide to bring the concentration of sodium *p*-nitrophenoxide in the solution to be measured within the limits of 0.003 to 0.010 mg. per ml. The dilution necessary will vary with the sample.

DETERMINATION OF PARATHION IN ETHER SOLUTION. Standardization of Sodium Nitrite. Weigh accurately 0.4 to 0.45 gram of anhydrous sulfanilic acid (the purity of which has been checked by a nitrogen determination) into a 400-ml. tall-form beaker. Add 80 ml. of water, 10 ml. of concentrated hydrochloric acid, 30 ml. of glacial acetic acid, and 5 grams of sodium bromide. Cool the mixture to 0° to 10° C. by the addition of clean shaved ice and place under mechanical stirring. Titrate at 0° to 10° C. with 0.1 *N* sodium nitrite as rapidly as the spot test will permit. Near the end point add the nitrite in 4-drop portions.

Spot Test. Dip a glass rod into the solution to be tested and then touch the rod quickly to a piece of potassium iodide–starch paper. The end point is reached when an intense blue-black color appears immediately and can be obtained repeatedly during a 1-minute period without further addition of the nitrite.

$$\text{Normality of sodium nitrite} = \frac{\text{grams of sulfanilic acid} \times 1000}{\text{ml. of } NaNO_2 \times 173.2}$$

Analysis of Ether Solution for Parathion. Add 35 ml. of the acetic-hydrochloric acid solution to the ether solution of parathion which has been transferred to a 400-ml. beaker. Add 1 gram of zinc dust and cover the beaker with a watch glass. Heat the solution gently on a steam bath until most of the ether has evaporated and the solution is colorless. Add 10 ml. of concentrated hydrochloric acid to complete the solution of the zinc dust. Cool the solution, wash down the beaker and watch glass with 100 ml. of water, and add 5 grams of sodium (or potassium) bromide. Cool the mixture to 0° to 10° C. by the addition of clean shaved ice (about 100 grams) and place under mechanical stirring. Titrate at 0° to 10° C. with standardized 0.1 *N* sodium nitrite as rapidly as the spot test will permit. About 20 to 30 ml. will be required. Near the end point add the nitrite in 4-drop portions.

$$\% \text{ parathion} = \frac{\text{ml. of sodium nitrite} \times \text{normality of sodium nitrite} \times 29.13}{\text{grams of sample}}$$

ANALYSIS OF DUST PREPARATIONS

Transfer a weighed sample of dust or wettable powder to an extraction thimble and extract with 150 ml. of ethyl ether in a Soxhlet apparatus for 1 hour. Transfer the ether extract to a 250-ml. separatory funnel and proceed with the analysis as described above.

The sample size will vary with the concentration of the dust: 10% = 6.75 grams; 15% = 4 to 5 grams; 25% = 2.5 to 3.5 grams.

DISCUSSION

The results of the analysis of parathion, purified by a combination of countercurrent adsorption and crystallization procedures (*7*), are listed in Table I. These results show that the recovery of known amounts of parathion is within ±0.5%, the accuracy expected being limited by the fact that increments of 0.2 ml. of 0.1 *N* sodium nitrite must be added to produce a decided end point. The satisfactory recoveries also indicate that sodium carbonate extraction does not remove appreciable parathion from ether solution. This was further checked by extracting the sodium carbonate solution with benzene, and determining parathion in the benzene extract by the method of Averell and Norris (*2*). The benzene extract contained less than 0.2 mg. of parathion.

Results of analyses of mixtures of purified parathion and *p*-nitrophenol are shown in Table II. The recovery of parathion is well within the accuracy of the method; the recovery of *p*-nitrophenol in the range in which it might be found in commercial parathion is satisfactory. Technical parathion in which *p*-nitrophenol is present in fairly high concentrations would generally be unsatisfactory for use and therefore a method with an accuracy better than that expected of a colorimetric procedure is not essential for control work. The reproducibility of the method is shown also in Table III, where results of the analysis of random samples of technical parathion are listed.

Table II. Analysis of Mixtures of Parathion and *p*-Nitrophenol

Parathion, %			*p*-Nitrophenol, %	
Added	Found	Recovery, %	Added	Found
100.0	99.5	99.5	0.029	0.033
99.9	99.7	99.8	0.083	0.083
99.9	100.1	100.2	0.077	0.068
99.7	99.6	99.9	0.29	0.30
99.3	99.5	100.2	0.67	0.71
95.0	94.9	99.9	4.9	5.1
93.4	93.0	99.6	6.6	7.1
92.4	92.4	100.0	7.6	7.0
93.3	93.0	99.7	6.6	6.4
90.8	91.0	100.2	9.1	8.5
92.6	92.8	100.2	7.3	7.1

Table III. Analysis of Technical Parathion

Sample No.	Parathion, %	*p*-Nitrophenol, %
1	97.8	0.17
	97.7	0.18
	98.0	0.17
	98.0	0.18
	97.7	0.17
	98.1	0.18
	Av. 97.9	
2	98.8	0.10
	98.7	0.12
	98.5	0.10
	98.6	0.11
	98.3	0.12
	98.8	0.11
	Av. 98.6	
3	97.2	0.95
	97.2	0.95
	97.3	0.94
	96.8	0.96
	96.7	0.95
	96.7	0.99
	Av. 97.0	

Table IV. Analysis of Synthetic Wettable Powder Formulations

Parathion, %		*p*-Nitrophenol, %	
Added	Found	Added	Found
20.5	20.5	0.82	0.74
21.8	21.7	1.4	1.4
21.2	21.0	0.77	0.72
21.1	20.9	1.2	1.2
19.5	19.5	1.3	1.3
24.0	23.8	0.99	0.93
21.1	21.0	1.3	1.2
20.6	20.5	0.94	0.91
21.7	21.8	0.68	0.64
22.6	22.6	0.68	0.64
21.4	21.5	1.1	1.1
27.0	27.0	0.45	0.43
25.4	25.2	0.11	0.10
27.8	27.8	0.073	0.066
21.5	21.6	0.22	0.22

To test the application of this procedure to the analysis of wettable powders and dusts, synthetic preparations were made by adding weighed amounts of purified parathion and *p*-nitrophenol to the inert filler. Ether was added and the mixture was stirred carefully to bring about uniform mixing of parathion, *p*-nitrophenol, and carrier. After evaporation of the ether, the dry dust was transferred to an extraction thimble and analyzed as described above. The results of these analyses are shown in Table IV.

The separation of parathion from *p*-nitrophenol is desirable, not only to determine both constituents on the same sample but also to avoid having *p*-aminophenol present during the titration of *O,O*-diethyl *O*-*p*-aminophenyl thiophosphate with sodium nitrite. Because *p*-aminophenol is diazotized more slowly than the amino derivative of parathion, the starch-iodide end point is not so sharp when *p*-aminophenol is present. The determination of *p*-nitrophenol is advisable, as it has been shown that excessive amounts of this impurity in the insecticide will cause injury to some plant tissues (*1*, *5*).

No distinction would be made in this method between parathion and other aromatic nitro or amino compounds. Theoretically possible impurities of this nature are the oxygen analog of parathion, diethyl *p*-nitrophenyl phosphate; the *S*-ethyl isomer, *O,S*-diethyl *O*-*p*-nitrophenyl phosphate; and the "bis compound," *O*-ethyl *O,O*-bis(*p*-nitrophenyl) thiophosphate. There is no evidence, however, that these or other nitro or amino compounds would be present in significant amounts.

ACKNOWLEDGMENT

The authors are indebted to E. F. Williams for supplying highly pure parathion used in this investigation.

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Determination of Titanium(III) in Titaniferous Slags

L. E. MACCARDLE AND E. R. SCHEFFER
National Lead Co., Titanium Division, South Amboy, N. J.

IN slags containing iron and titanium, varying amounts of titanium(III) and iron(II) occur. Of the methods proposed the determination of titanium(III) (*1*, *2*, *4*), those performed without the benefit of an inert atmosphere such as carbon dioxide gave erratic results due to air oxidation of the sample, which takes place rapidly even at room temperature. The use of an inert atmosphere requires special care and equipment, and the procedure leaves much to be desired. The values obtained by previous methods where a carbon dioxide atmosphere was used indicate errors due to the incomplete solution of the sample.

If the titanium(III) and iron(II) can be oxidized under controlled conditions with complete solution of the sample, accurate analyses can be performed. In this procedure the titanium(III) and iron(II) react with the standardized vanadium pentoxide immediately upon being dissolved, in accordance with the following equation:

$$Ti^{+++} + Fe^{++} + 2V^{+++++} \longrightarrow Ti^{++++} + Fe^{+++} + 2V^{++++}$$

The addition of hydrofluoric acid permits complete solution without interference with the analysis. The excess vanadium pentoxide is determined by titration with standard ferrous ammonium sulfate. If titanium(III) is present in any slag, all the iron must be in the ferrous state. The vanadium pentoxide reacts with both iron(II) and titanium(III). The iron can be determined by any conventional method, and the amount of titanium(III) calculated.

PREPARATION AND STANDARDIZATION OF SOLUTIONS

Vanadium pentoxide, approximately 0.36 *N*. Dissolve 50 grams of reagent grade vanadium pentoxide in 500 ml. of distilled water and 150 ml. of concentrated sulfuric acid. Filter if necessary, employing glass wool. Cool and dilute to 1000 ml. Standardize with standard ferrous ammonium sulfate solution.

Ferrous ammonium sulfate, 0.1 *N*. Dissolve 39.22 grams of ferrous ammonium sulfate hexahydrate in 500 ml. of distilled water and 10 ml. of concentrated sulfuric acid. Cool, dilute to 1000 ml., and store in a brown glass bottle. Standardize with standard potassium permanganate.

PROCEDURE

Transfer 0.5 gram of slag into a 100-ml. platinum dish. Add from a pipet 10 ml. of the standardized vanadium pentoxide solution, partially cover with a watch glass, and slowly add 30 ml. of 1 to 3 sulfuric acid. Heat to boiling and cautiously add 10 ml. of hydrofluoric acid.

When the sample is dissolved, allow it to cool and transfer the contents into an 800-ml. beaker containing 300 ml. of water and 10 ml. of phosphoric acid. Add 5 drops of barium diphenylamine sulfonate indicator and titrate to a blue-green end point with ferrous ammonium sulfate.

The iron was determined separately by a volumetric dichromate procedure (*3*). However, after solution and oxidation of the sample, the iron can be determined by any conventional method.

EXPERIMENTAL

Standard samples of titanium(III) oxides were prepared by the reduction of titanium dioxide with hydrogen at high temperatures. The titanium(III) values were obtained by ignition of the oxide in the presence of air. These samples were analyzed and compared to the ignition values. The types of results obtained are given in Table I.

Table I. Synthetic Reduced Titanium Oxides

Sample	Ti(III), % In mixed oxides by ignition	Ti(III), % By V_2O_5 method
1	5.5	5.9
2	48.1	48.4

Table II. Synthetic Slag Samples

Samples	Ti(III) Added, Gram	Ti(III) Found, Gram
	Solutions	
1	0.028	0.027
2	0.082	0.086
3	0.110	0.109
4	0.165	0.165
	Ores with Iron(II)	
1	0.0205	0.0215
2	0.0205	0.0210
3	0.0820	0.0835
4	0.0820	0.0835

The synthetic slag solutions were prepared with ferrous ammonium sulfate and titanium(IV) sulfate to simulate slag ratios, to which titanous sulfate was added in varying proportions. Titanous sulfate solution was added to ores containing iron(II) to prepare the synthetic slag samples. The results, given in Table II, show the validity of the method in the presence of iron(II).

The precision of the method, based on a series of actual slag analyses, is ±0.05%.

CONCLUSION

The method for titanium(III) is simple and requires no special equipment. It gives better accuracy and precision than previous methods because of complete solubilization in the presence of a standard oxidizing agent, and elimination of air oxidation of the titanium(III) and iron(II). Positive errors are incurred if titanium carbide or large amounts of sulfide are present. Metallic iron must be absent.

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Determination of the Miticide, 2-Thenyl Salicylate

JEROME GOLDENSON AND SAMUEL SASS
Chemical Corps, Technical Command, Army Chemical Center, Md.

IN CONNECTION with work on 2-thenyl salicylate, a new effective miticide (*1*, *2*, *4*), methods of analysis were required which would be suitable for various development operations and applicable to clothing impregnated with the compound. A diversity of methods is desirable during development stages. The methods described in this paper include the bromination of salicylic acid and 2-thenyl alcohol after alkaline decomposition of the compound, saponification, and estimation of the violet color formed by salicylic acid with ferric alum solution, using a photoelectric colorimeter. These methods were found to be satisfactory for estimation of the miticide both free and in cloth.

APPARATUS

Soxhlet-type extraction apparatus with standard-taper Erlenmeyer flask.

Beckman quartz spectrophotometer, Model CUV.

Klett-Summerson photoelectric colorimeter with standardized colorimeter tubes and filter No. 54 (maximum transmittance 520 to 580 mμ) and standardized colorimeter tubes.

REAGENTS AND MATERIALS

2-Thenyl salicylate obtained from the Monsanto Chemical Co. was used as received.

Undyed and olive drab cotton herringbone twill cloth. Nacconol NR, sodium alkyl aryl sulfonate used as detergent in laundering cloth, made by National Aniline and Chemical Corp., New York, N. Y. Fixatives such as chlorinated paraffin (U. S. Army Specification 4-503-127B). Plasticizers such as dioctyl phthalate, technical grade, made by Carbide and Carbon Chemicals Division, Union Carbide & Carbon Corp.

Reagents for Bromination Method. Ethyl ether, C.P. Potassium hydroxide solution, approximately 0.1 *N*. Hydrochloric acid, C.P. concentrated. Potassium bromate–potassium bromide solution of 3.5 grams of potassium bromate, C.P., and 13.0 grams of potassium bromide, C.P., made up to 1 liter with water. Potassium iodide solution, 10%. Sodium thiosulfate solution, 0.1 *N*. Starch indicator solution, 1%.

Reagents for Ferric Alum Colorimetric Method. Ferric alum solution, prepared as follows: Dissolve 4.8 grams of ferric ammonium sulfate dodecahydrate in 50 ml. of water, add 0.1 ml. of 30% hydrogen peroxide, and boil until the peroxide is destroyed. Add 5 ml. of 1 to 1 sulfuric acid solution and heat until the solution is clear. Cool, dilute to 100 ml. with water, and adjust to a pH of 1.4 with 10% potassium hydroxide solution, using a pH meter. This solution will keep indefinitely when stored in a glass-stoppered bottle.

DEVELOPMENT OF METHODS

Saponification Method. It was found that 2-thenyl salicylate may be extracted from the cloth and saponified in one operation by placing alcoholic potassium hydroxide in the flask of a Soxhlet extraction apparatus and adding sufficient alcohol to the extraction thimble to ensure satisfactory siphoning. Recoveries of 100.0 and 99.8% were obtained with known amounts of the compound in undyed cotton herringbone twill cloth. Chlorinated paraffin, when used as a fixative, gives high results in the method, Nacconol NR interferes slightly, and the method cannot be used in the presence of plasticizers such as dioctyl phthalate.

The electrometric titration curve relating pH and milliliters of 0.1 *N* hydrochloric acid used to back-titrate the excess alkali indicated that the greatest change of pH took place at about 8.5.

Procedure. 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 30 mg. of the compound) in the thimble of a Soxhlet extraction apparatus, and add 50.00 ml. of 0.1 *N* alcoholic potassium hydroxide to the flask. Add alcohol to the thimble in an amount about 10 ml. in excess of that required to fill the thimble portion of the extractor. Extract and saponify for 2 hours. Remove the flask from the apparatus, cool, add 50 ml. of distilled water and a few drops of

phenolphthalein indicator, and titrate to a colorless end point with 0.1 N hydrochloric acid. Run a blank determination under the same conditions with unimpregnated cloth.

If the solution is colored by cloth impurities, a mixed indicator of 0.05% thymol blue and 0.15% phenolphthalein in 50% alcohol may give a better end point. For highly colored solutions, it is recommended that the titration be done electrometrically to a pH of 8.5.

Calculation

$$\% \text{ 2-thenyl salicylate} = \frac{(b - s) \times N \times 23.527}{w}$$

where b = ml. of blank hydrochloric acid titration
s = ml. of sample hydrochloric acid titration
N = normality of hydrochloric acid
w = weight of sample, grams

NOTE. A correction may be made for the interfering effect of chlorinated paraffin by using the Volhard method to determine the liberated chloride in the solution after the titration with standard acids, and making a correction for the amount of sodium hydroxide consumed by the chlorinated paraffin. In this case standard sulfuric acid must be used in place of the standard hydrochloric acid.

Bromination Method. Attempts to brominate 2-thenyl salicylate directly by published methods (*3*, *7*) in alcohol, carbon tetrachloride, or acetic acid solutions were unsuccessful; variable results not satisfactory for quantitative application were obtained. When the compound was first decomposed by alkali to salicylic acid and thenyl alcohol, followed by a bromination time of 20 minutes, recoveries of 100.0 and 100.2% were obtained. It was found that 7 moles (14 atoms, 6 for salicylic acid and 8 for thenyl alcohol) of bromine were required for each molecule of 2-thenyl salicylate. If a shorter bromination time was used, low results were obtained.

In applying the method to cloth impregnated with 2-thenyl salicylate, the compound was extracted with ethyl ether, followed by evaporation of the ether, decomposition with 0.1 N potassium hydroxide solution, and bromination with potassium bromate and potassium bromide solution. Several trials were run using known amounts of the compound impregnated in undyed and olive drab herringbone twill cloth. The results were uniform and the recoveries were better than 99% with samples containing as little as 2 mg. No interference was experienced from chlorinated paraffin, dioctyl phthalate, and pure-finish and olive drab herringbone twill cloth. However, a very slight blank titration was obtained with sized cloth, and for optimum accuracy a blank correction should be made.

Procedure. Cut the cloth sample into 1-cm. squares and mix. Extract a weighed sample of cloth containing at least 5 mg. of 2-thenyl salicylate for 1.5 hours with ethyl ether in a Soxhlet-type extractor with a paper thimble. (If Soxhlet equipment is not available, the cloth may be heated directly with the potassium hydroxide solution and the extract separated by filtration. Blanks will be slightly higher if this is done.) Evaporate the ether carefully from the extract in the flask of the extractor and add 25 ml. of aqueous 0.2 N potassium hydroxide solution. (If a sample containing about 150 mg. or more of 2-thenyl salicylate is used, 0.5 N in place of 0.2 N potassium hydroxide solution should be used, and an aliquot portion should be used in place of the entire extract.)

Attach a reflux condenser to the flask and heat to boiling until the compound is saponified and in solution (about 30 minutes). Cool, transfer to a 500-ml. iodine flask, add a measured amount of potassium bromate–potassium bromide solution (25 to 35 ml., depending on the amount needed to have at least 2 to 5 ml. excess over that required for bromination), and add 1 ml. of concentrated hydrochloric acid for each 25 ml. of solution (if the volumes of potassium hydroxide and potassium bromate–potassium bromide solution are changed, use amounts of hydrochloric acid to have an acidity between 0.7 and 0.8 N). Insert the stopper quickly and shake intermittently for 20 minutes. Add 10 ml. of 10% potassium iodide solution, taking care to avoid loss of bromine while the stopper is being lifted. Wash down the stopper and the sides of the flask with water and titrate the liberated iodine with 0.1 N sodium thiosulfate solution, using starch indicator at the end of the titration. Run a cloth blank under the same conditions. The difference between the titration volume of the blank and that of the sample after bromination is the net titration.

Calculation

$$(\%) \text{ 2-thenyl salicylate} = \frac{t \times N \times 1.6805}{w}$$

where t = ml. of net sodium thiosulfate titration
N = normality of sodium thiosulfate
w = weight of sample, grams

Results obtained on pilot plant–impregnated batches of cloth, some of which had been laundered varying numbers of times, are given in Table I.

Colorimetric Ferric Alum Method. This method is described in the literature (*6*) for salicylic acid, and when applied to the estimation of 2-thenyl salicylate involved only the additional step of decomposing the compound to salicylic acid by alkali and a slight modification of the procedure. Mehlig (*5*) adapted this method to determining iron by means of a spectrophotometer. In the work described here, the method has been adapted to the determination of salicylic acid by means of a photoelectric colorimeter.

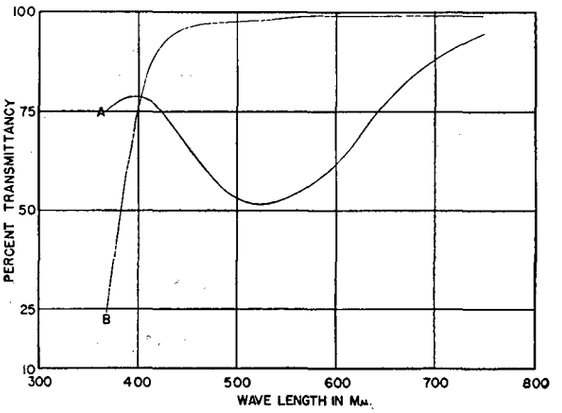

Figure 1. Absorption Spectra

A. **Salicylic acid–ferric alum solution**
B. **Ferric alum blank solution**

The absorption spectrum of the colored solution formed by salicylic acid (concentration of 0.05 gram of 2-thenyl salicylate per liter) with ferric alum, as well as the spectrum of the blank ferric alum solution, as determined with a Beckman spectrophotometer, is plotted in Figure 1, showing that the maximum absorption for this colored solution is in the range of 520 to 540 mμ. The Klett-Summerson photoelectric colorimeter with filter 54, which transmits in the range of 520 to 580 mμ was used for the quantitative estimations. The curve relating colorimeter readings with concentration of 2-thenyl salicylate obtained by application of this method to known solutions was a straight line up to a concentration of 0.09 gram of 2-thenyl salicylate per liter.

The method was found very satisfactory for the determination of small amounts of 2-thenyl salicylate. Large amounts involved the error of large dilution. No interferences were experienced in this method in analyzing undyed pure-finish or sized cloths and olive drab cloth. Chlorinated paraffin and dioctyl phthalate do not interfere.

Results obtained by the colorimetric method in comparison with the bromination method on pilot plant–impregnated batches of cloth, some of which had been laundered varying numbers of times, are given in Table I.

Procedure. Cut the cloth sample into 1-cm. squares and mix. Extract a weighed sample and saponify the extract as directed in the bromination procedure. Neutralize the resultant alkaline solution with dilute hydrochloric acid to a pH of about 7 (a drop of phenolphthalein solution may be used as an indicator) and make up to a known volume with distilled water. Dilute por-

tions of this solution to such a volume that 0.004 to 0.08 mg. per ml. of the 2-thenyl salicylate will be present. If the 2-thenyl salicylate concentration is completely unknown, prepare several dilutions until the right range is obtained. In each case, place 10 ml. of the diluted solution in a calibrated Klett-Summerson tube, add 0.20 ml. of the ferric alum solution, mix well, and allow to stand for at least 1 minute (if the solution is cloudy, filter into the Klett tube). (The color developed after about 1 minute under these conditions has been found to be stable for at least 5 days in the absence of strong sunlight.) Take the reading in a Klett-Summerson photoelectric colorimeter, using green filter 54. Prepare a curve relating colorimeter reading with 2-thenyl salicylate concentration using known solutions treated as described above, and make the quantitative determination of the unknown by applying the reading obtained.

DISCUSSION

The bromination method was found to be rapid and accurate for amounts down to 5 mg. of 2-thenyl salicylate. No interference was experienced with undyed pure-finish or olive drab cotton cloth, fixatives such as chlorinated paraffin, or plasticizers such as dioctyl phthalate. A slight blank correction is necessary with sized-cloth samples. The colorimetric method is also rapid and accurate for the estimation of amounts down to 0.05 mg. of 2-thenyl salicylate. No interferences are experienced from undyed or olive drab pure-finish or sized cloth, chlorinated paraffin, and dioctyl phthalate. The saponification method was used only as a check method and is accurate for amounts down to about 30 mg. of the miticide. Nacconol NR interferes slightly; a special correction must be made in the presence of chlorinated paraffin; and the method is not applicable when dioctyl phthalate is present.

Table I. Determination of 2-Thenyl Salicylate in Cloth Impregnated in a Pilot Plant

Sample No.	2-Thenyl Salicylate, %	
	Bromination procedure	Colorimetric ferric alum method
T-0	5.49	5.55
T-7	5.49	5.53
T-18	5.92	5.81
TOL	6.35	6.35
T1L	4.25	4.15
T2L	2.61	2.54
T3L	1.52	1.46
T4L	0.95	0.93
T5L	0.38	0.37
T6L	0.26	0.27
T7L	0.12	0.12
T8L	0.07	0.08
T9L	0.06	0.07
T10L	<0.05	0.03

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Determination of Glucose in Presence of Maltose

Rapid and Convenient Semimicromethod

LOUISE LANG PHILLIPS AND M. L. CALDWELL
Columbia University, New York, N. Y.

IN THE investigation of amylase action, it is often important to determine small concentrations of glucose in the presence of maltose and other reducing products. The method developed to meet this need is essentially a modification of the method of Zerban and Sattler (*7*), but adjusted for the determination of much smaller concentrations of glucose. Instead of 20 to 80 mg. of glucose in volumes of 20 ml., it permits the accurate determination of 2 to 6 mg. of glucose in 2 ml. of sugar solutions or of starch hydrolyzates in the presence of maltose and other products of amylase action. The present report introduces no new theoretical principles, but unites in one convenient rapid procedure conditions that have been found essential to accurate and precise results.

The method of Zerban and Sattler (*7*) and that reported here employ the Soxhlet modification of Fehling's copper solution, and an excess of sodium acetate (*6*). The cuprous oxide formed in the reduction is determined by the iodometric method of Shaffer and Hartman (*5*). The method depends primarily upon the equations:

$$KIO_3 + 5KI + 3H_2SO_4 \longrightarrow 3K_2SO_4 + 3I_2 + 3H_2O$$

$$KIO_3 + 5KI + 9H_2SO_4 + 3Cu_2O \longrightarrow 6CuSO_4 + 3K_2SO_4 + 6HI + 6H_2O$$

The difference between the blank determination of the iodine released (by thiosulfate titration) and the unknown, in which some of the potassium iodate has been used to oxidize the cuprous oxide formed, is a measure of the glucose present in the mixture.

REAGENTS

Copper Sulfate (Soxhlet modification of Fehling's solution). Dissolve 69.28 grams of copper sulfate pentahydrate in water and dilute to 1 liter. Use large clear crystals showing little or no efflorescence.

Sodium Acetate Trihydrate, reagent grade. Dissolve 500 grams in about 800 ml. of hot distilled water, cool, and dilute to 1 liter. This solution should give pH values of 8.7 to 8.8. If the solution gives lower pH values it should be discarded.

Potassium Iodide-Iodate. Dissolve 5.400 grams of reagent grade potassium iodate and 60.0 grams of reagent grade potassium iodide in about 500 ml. of distilled water, add 0.25 gram of sodium hydroxide, and dilute to exactly 1 liter.

Sulfuric Acid, approximately 4 *N*. Add 114 ml. of concentrated sulfuric acid to 500 ml. of distilled water, cool, and dilute to 1 liter.

Potassium Oxalate. Dissolve 330 grams of potassium oxalate crystals in 1 liter of hot distilled water (saturated solution). Cool.

Sodium Thiosulfate, 0.01 *N*. Dilute 0.1 *N* solution prepared by dissolving 24.8 grams of reagent grade sodium thiosulfate pentahydrate and 3.0 grams of sodium tetraborate in 1 liter of water. The dilute solution should be freshly prepared and standardized.

Dextrose, Bureau of Standards, 1% solution for preparing calibration charts.

Maltose Hydrate, 2% solutions for preparing calibration curves.

Table I. Data for Calibration Curves

Glucose[a], Mg./2 Ml.	Maltose, Mg. per 2 Ml. None	5	10	20
	Titer of 0.01 *N* Sodium Thiosulfate, Ml.			
	A. Maltose[b] 1			
1	3.37	5.19	7.17	10.37
2	7.38	8.93	10.55	12.70
3	11.24	12.19	13.28	14.59
4	13.80	14.56	15.45	16.59
5	15.83	16.40	16.97	17.91
6	17.43	17.90	18.57	19.55
	B. Maltose[c] 2			
1		5.38	7.21	9.99
2	7.65	9.26	10.61	12.36
3	11.56	12.22	13.23	14.26
4	14.28	14.66	15.47	16.38
5	16.06	16.49	17.06	17.66
6	17.53	17.90	18.60	19.11
	C. Maltose[d] 3			
2	7.65	...	10.30	11.65
3	11.56	...	13.18	14.17
4	14.28	...	15.04	15.87
5	16.06	...	...	...
6	17.53	...	18.24	18.91

[a] Glucose, National Bureau of Standards.
[b] Maltose, $[\alpha]_D^{25} = 129.9$. Reducing value 93% determined by iodometric method (*1*).
[c] Maltose $[\alpha]_D^{25} = 130.1$. Reducing value 95% determined by iodometric method (*1*).
[d] Maltose, highly purified, prepared from starch by action of β-amylase and recrystallized; $[\alpha]_D^{25} = 131.25$. Reducing value 98.5% determined by iodometric method (*1*).

PROCEDURE

Portions of 2 ml. of the sugar solution or the hydrolyzates to be examined (or smaller volumes diluted to a total volume of 2 ml.) are added to 1 ml. of the copper sulfate solution and 2 ml. of the sodium acetate solution in a test tube. Tubes thus prepared and similar blank tubes containing 2 ml. of water instead of the sugar solution are covered with glass bulbs and placed in briskly boiling water for 20 minutes. It is important that the tubes be placed in a rack, so that they do not touch the bottom or sides of the container and that the level of the boiling water be above that of the solution in the test tubes. The test tubes should also be of the same size.

At the end of 20 minutes the tubes are cooled in running water for 4 minutes. The following solutions are then added rapidly: 3 ml. of the potassium iodide-iodate solution, 1 ml. of the dilute sulfuric acid solution, and 2 ml. of the potassium oxalate solution.

All reagents are added rapidly to one tube at a time and mixed before going on to the next tube. The solutions are then mixed thoroughly and allowed to stand at room temperature for 30 minutes or longer with occasional additional mixing. All traces of red cuprous oxide or white cuprous iodide precipitate must be dissolved. The greenish blue cupric oxalate precipitate which sometimes forms does not interfere.

After standing at room temperature for 30 minutes or longer, the contents of the tubes are transferred to 125-ml. Erlenmeyer flasks and the tubes are washed six times with 2.5-ml. portions of distilled water. The resulting solutions are then titrated with 0.01 *N* sodium thiosulfate.

CALIBRATION CURVES

With solutions of pure glucose, the difference between the titer of sodium thiosulfate for the blank and for the glucose solution is due to the reducing action of glucose under the conditions employed. Therefore, a calibration curve is set up for the values obtained with solutions containing known weights of pure glucose and the reagents and equipment employed. For this curve, the milliliters of 0.01 *N* thiosulfate are plotted against milligrams of glucose per 2 ml. of glucose solution. Solutions containing 0.5 to 8 mg. of glucose per 2 ml. are used.

Maltose causes a small but often significant reduction of the cupric ion in copper sulfate, even in the presence of acetate. Therefore, it is necessary also to set up glucose-maltose calibration curves for solutions containing known small weights of maltose in addition to known weights of glucose. When plotted as above, a separate glucose-maltose calibration curve is obtained for each concentration of maltose with glucose. The calibration curves used here were based upon solutions containing 0.5 to 8 mg. of glucose and 5, 10, or 20 mg. of maltose hydrate.

It is desirable for each operator to prepare calibration curves for use with the reagents and equipment at hand to compensate for differences in technique and in the quality of the reagents.

CORRECTION FOR REDUCING ACTION OF MALTOSE

With unknown sugar solutions the difference between the titer of sodium thiosulfate for the blank and that given by the sugar solution may be influenced by the presence of maltose. Therefore, this difference represents the apparent rather than the true glucose. The value for this apparent glucose is read from the glucose calibration curve and must be corrected for any maltose in the sugar solution.

In order to make this correction, the total reducing value of the sugar solution is determined by an iodometric method (*1*). This value is stoichiometric and includes both glucose and maltose. It is calculated to its glucose equivalents. The apparent weight of maltose is then determined in turn by taking the difference between the total reducing value as glucose equivalents and the apparent weight of glucose taken from the glucose calibration curve. This difference, expressed as glucose equivalents, must be multiplied by two to convert it back to maltose on which the glucose-maltose calibration curves are based.

The true glucose value of the solution is then read from the glucose-maltose calibration curve representing the weight of admixed maltose nearest to the apparent value for maltose obtained above or, when necessary, by interpolation between two glucose-maltose curves.

If the glucose value thus obtained differs significantly from the apparent value obtained above, it may be necessary to recalculate the apparent maltose value on the basis of the new glucose value and then to redetermine the true glucose value from the recalculated maltose value.

RESULTS

Scope and Accuracy of Method. The average data given in Table I were used to prepare calibration curves for glucose alone and in the presence of known weights of maltose. These data were plotted on large size graph paper, from which the glucose could be read easily to 0.01 mg.

The three samples of maltose employed in this work did not differ markedly from each other in their optical activities. However, the reducing values obtained by the iodometric method (*1*) differed considerably. Table I shows that the three samples of maltose gave different reducing values with copper acetate in the presence of the same concentrations of the same glucose under similar conditions. Two samples of maltose, 1 and 2, were found later by amylase action (*3*) to be contaminated with small concentrations of both glucose and dextrins in proportions to compensate for each other in measurements of optical rotation. Therefore, subsequent work was carried out with glucose-maltose calibration curves based on measurements made with the third highly purified maltose that had been obtained by the action of beta-amylase on starch.

Comparison of the data for glucose alone given in Table I, A and B, shows different reducing values for the glucose. These data were obtained by the same operator with the same pure glucose and the same equipment but with different samples of sodium acetate. Both samples of sodium acetate gave values of pH 8.7 to 8.8 for 50% solutions. These data emphasize the importance of the recommendation that each operator prepare his own calibration curves.

The data given in Table II represent a study of the influence of differences in the proportions of glucose to maltose in the sugar solutions upon the accuracy of the method. These solutions contained different concentrations of glucose in the presence of 4 and 16 mg. of maltose hydrate per 2 ml. These two levels of maltose were used to determine whether there is a greater error when large quantities of maltose are present than when the pro-

portion of glucose to maltose is more nearly equal. With 4 mg. of maltose hydrate, the average error was 0.08 mg. of glucose per 2 ml. or 1.6%. With 16 mg. of maltose hydrate, the average error was 0.11 mg. of glucose per 2 ml. or 2.7%.

Comparison of Chemical Method with Selective Fermentation with Yeast. The data summarized in Table III compare the results obtained by the method described here with those obtained by selective fermentation with yeast No. 2019, as recommended by Schultz *et al.* (*4*). With solutions of known concentrations of glucose, the method described gave an average error of 0.19 mg. of glucose per 2 ml. in solutions containing from 0 to 16 mg. of glucose per 2 ml. The maximum error was 0.42 mg. per 2 ml. The yeast fermentation method, using Fleischmann's yeast No. 2019, gave an average error of 0.23 mg. of glucose per 2 ml. on the same fourteen sugar solutions. With the fermentation method, the maximum error was 0.58 mg. per 2 ml. The two methods were also compared with hydrolyzates obtained at 40° from 1% Lintner's soluble potato starch or 1% maltose by the action of a glucose-forming amylase (*3*) produced by the mold, *Rhizopus delemar* (*2*). This comparison is summarized in Table IV, which shows an average difference of 5.0% when the substrate was starch, and 10.3% when maltose was the substrate.

The data given in Tables II and III for solutions of known concentrations of glucose or of glucose and maltose show that glucose can be determined by the method described here with an error of 0.1 to 0.2 mg. of glucose per 2 ml. in glucose solutions that contain 2 to 6 mg. of glucose per 2 ml. and in the presence of 20 mg. or less of maltose.

DISCUSSION

Certain precautions in the use of the copper reduction method should be observed.

Table II. Influence of Proportion of Maltose to Glucose upon Accuracy of Method

	Maltose Hydrate Added, Mg. per 2 Ml.					
	4			16		
Glucose Added, Mg./2 Ml.	Glucose Recovered, Mg./2 Ml.	Difference, Mg./2 Ml.	Error, %	Glucose Recovered, Mg./2 Ml.	Difference, Mg./2 Ml.	Error, %
2	1.97	0.03	1.5	2.09	0.09	2.5
2	1.97	0.03	1.5	2.12	0.12	6.0
3	2.91	0.09	3.0	2.96	0.12	1.3
3	2.99	0.01	0.3	2.93	0.07	2.3
4	4.08	0.08	2.0	3.86	0.14	3.6
4	3.94	0.06	1.5	3.90	0.10	2.5
4	..	...	...	3.84	0.16	4.0
5	4.95	0.05	1.0	4.75	0.25	5.0
5	4.88	0.12	3.0	4.79	0.21	4.2
6	6.21	0.21	3.5	6.02	0.02	0.3
6	..	...	...	6.05	0.05	0.8
6	..	...	...	6.10	0.10	1.7
Av.	..	0.08	1.6	..	0.11	2.7

Table III. Comparison of Chemical Method and Selective Fermentation with Yeast for Determination of Glucose

(Mg. per 2 ml.)

Solution Examined				Chemical Method		Selective Fermentation with Yeast[a]	
Sugar solution No.	Starch[b] substrate	Maltose	Glucose	Glucose recovered	Error	Glucose recovered	Error
1	0	21.06	0	0.06	+0.06	0.42	+0.42
2	0	18.90	2	1.75	−0.25	2.16	+0.16
3	0	16.80	4	3.58	−0.42	4.20	+0.20
4	8	4.2	2	1.91	−0.09	1.81	−0.19
5	8	4.2	4	3.64	−0.36	3.62	−0.38
6	8	4.2	8	7.80	−0.20	7.42	−0.58
7	8	0	0	0.13	+0.13	0.24	+0.24
8	0	13.44	8.0	7.98	−0.02	8.02	+0.02
9	0	16.85	4.8	4.53	−0.27	4.74	−0.06
10	0	10.16	10.0	9.76	−0.24	9.68	−0.32
11	0	5.04	16.0	15.76	−0.24		
12	4.0	10.16	6.4	6.16	−0.24	6.25	−0.15
13	8.0	10.16	3.2	3.31	+0.09	3.13	−0.07
14	16.0	5.04	0	0	0	−0.18	−0.18
Av.					0.19		0.23

[a] Yeast 2019, kindness of A. S. Schultz and Fleischmann Laboratories.
[b] Lintner's soluble potato starch adjusted to 0.01 *M* acetate, pH 4.5 (*3*).

Table IV. Comparison of Chemical Method and Yeast Fermentation upon Analysis of Amylase Hydrolyzates for Glucose

	Glucose Produced by Amylase, Mg. per 2 Ml. of Hydrolyzate			
	Starch[a] as Substrate		Maltose[a] as Substrate	
Hydrolysis Time, Hours	Chemical method	Yeast fermentation	Chemical method	Yeast fermentation
1	5.84	5.23	2.10	2.25
2	9.58	9.31	3.00	3.65
3	11.10	11.21	4.46	4.74

[a] A glucose-forming amylase reacted with 1% Lintner's soluble potato starch or with 1% maltose, pH 4.5; 0.01 *M* acetate and 40° (*3*).

It is absolutely essential that a good grade of sodium acetate be used. The 50% solution of sodium acetate should give a value of approximately pH 8.8. Some solutions of so-called reagent grade sodium acetate give values as low as pH 6.7 at 50% concentrations. Such solutions are entirely unsatisfactory.

Thorough mixing of reagents in the test tubes is essential both before boiling and after the second group of reagents has been added. Insufficient mixing before boiling will give incomplete precipitation of cuprous oxide. Insufficient mixing after addition of the second group of reagents permits the formation of difficultly soluble cuprous iodide which shifts the equilibrium of the reaction:

$$2Cu^{++} + 4I^- \rightleftharpoons 2CuI + I_2$$

to the right and thus prevents an accurate determination of the amount of cuprous ion formed by the reduction with glucose.

In any event, at least 30 minutes must be allowed after the second group of reagents has been added before the solutions are titrated. If less time is permitted for the reaction to come to equilibrium, a fading end point is obtained and the results cannot be checked.

Not more than 10 tubes should be prepared and boiled at one time. Otherwise, the time that elapses before the addition of all reagents is completed will cause poor results, which may be due to oxidation of the cuprous oxide by air before the reagents are added.

When the above precautions are observed, the use of the copper acetate reduction method in conjunction with the iodometric method (*1*) gives a convenient procedure for the determination of glucose in the presence of maltose, which compares favorably with the accuracy obtained by yeast fermentation. The chemical method is more easily and rapidly carried out than the fermentation methods which are in more general use, and it is more adaptable for routine activity measurements.

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Selection of Capillaries with Predetermined Characteristics for the Dropping Mercury Electrode

Nomogram and Directions

OTTO H. MÜLLER

State University of New York Medical Center at Syracuse, Syracuse, N. Y.

A PROBLEM facing everyone in polarography is the choice of the proper glass capillary for the dropping mercury electrode. It is known that to be suitable for analytical work, the latter should have a drop time between 2 and 6 seconds in 0.1 *N* potassium chloride at pressures between 30 and 70 cm. of mercury, but the selection of such a capillary has been a hit-or-miss proposition in which experience was the best guide. When it comes to laboratory-drawn capillaries, the selection will probably always remain that way unless the experimenter is sufficiently expert in their manufacture. However, in the case of the more widely used capillaries of uniform bore it is possible to calculate the exact dimensions of a capillary which will have a desired drop time at a desired pressure of mercury, so that experience is no longer necessary for its selection. This paper calls attention to this fact and gives the equations needed for the calculations as well as a nomogram for simplifying their application.

A capillary of uniform bore can be defined by its length and its internal diameter or radius. If a capillary is laboratory-drawn, this is not possible; either the bore is not uniform or the length of any existing uniform bore is not measurable. Nevertheless, such capillaries can still be characterized and compared by means of the radius of their orifice, ρ, and their capillary constant, κ, which represents that pressure of mercury necessary to cause the flow of 1 mg. of mercury per second through the capillary. Simple methods for the determination of these constants have been described (*1*). The following equation defines κ in terms of the length, l, of a capillary of uniform bore and of the radius of its orifice, ρ:

$$\kappa = 2.1567 \times 10^6 \, l/\rho^4 \qquad (1)$$

when κ is expressed in cm. sec. mg.$^{-1}$, l in centimeters, and ρ in microns (μ).

Consequently, by means of this equation, it is possible to calculate the dimensions of a capillary of uniform bore necessary to replace any laboratory-drawn capillary with bore of uncertain diameter, as long as the radius of its orifice, ρ, and its capillary constant, κ, are known.

Having thus a relationship generally applicable to any kind of capillary, we need to consider next the limits in the size of the bore of capillaries that are still suitable for ordinary polarographic work. For each capillary a critical pressure, P_c, can be found, below which mercury no longer drops from the capillary (*1*). This critical pressure is determined in the absence of electrical connections. It varies with the interfacial tension between the mercury and the electrolyte, σ, and it is a function of ρ, as shown by the following equation:

$$P_c = 2\sigma/g\mathrm{d}\rho \qquad (2)$$

where g is the gravitational constant and d is the density of mercury. A graph, illustrating this relationship, is given in Figure 1 for ready reference. It represents the equation $P_c = 573/\rho$ (where P_c is expressed in centimeters and ρ in microns) which holds for 25° C. and when $\sigma = 380$ dynes cm.$^{-1}$—i.e., when the capillary is dropping in a solution of 0.1 *N* potassium chloride in equilibrium with air. This solution is most commonly employed in the calibration of capillaries. Between experiments, the flow of mercury is usually arrested in distilled water, open to air, where $\sigma = 374$ dynes cm.$^{-1}$; the corresponding $P_c - \rho$ relationship is therefore practically identical with that shown in Figure 1. From this may be seen that pressures greater than 60 cm. of mercury are needed to produce any flow at all through capillaries with ρ values of less than 10 microns (or a diameter of < 20 microns). Such capillaries are therefore not feasible for practical polarography. On the other hand, capillaries with ρ values greater than 40 microns would continue flowing in potassium chloride solution or distilled water until the mercury pressure is diminished below 14 cm. This may be considered the upper limit in the size of useful capillaries for polarography, since capillaries of larger bore will use too much mercury in their operation, and require means for stopping the flow of mercury other than simple lowering of the reservoir. Hence, one can conclude that suitable capillaries for polarographic analyses must have internal diameters ranging from 20 to 80 microns. The most useful capillaries for all-around work will probably have diameters varying between 30 and 60 microns.

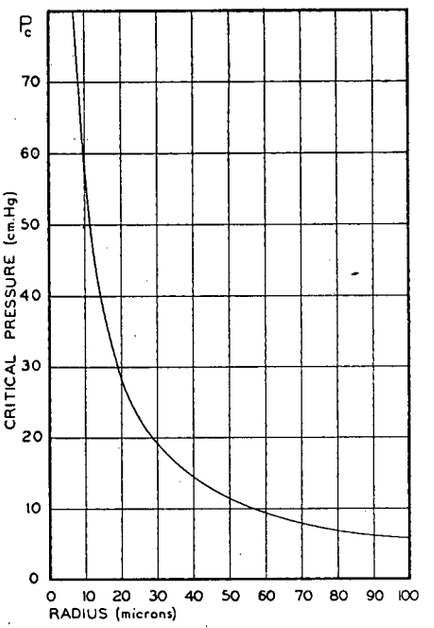

Figure 1. Relation of Critical Pressure to Radius of Orifice of Dropping Mercury Electrode in 0.1 *N* Potassium Chloride

Capillaries with a uniform bore within this range are commercially available as "marine barometer tubing." The bore of such capillaries, if not stated by the manufacturer, can be determined by means of a microscope equipped with a micrometer eyepiece; the smallest divisions of a hemocytometer will be found very helpful for calibration purposes. If microscopic equipment is not available, the critical pressure and drop-weight methods of Müller (*1*) can be used to determine the ρ value of a sample piece of the capillary tubing.

Given such capillaries of known uniform bore, one still needs to decide about a suitable length. This depends entirely upon the

wishes of the experimenter concerning the mercury pressure which he expects to use and the drop time which he desires. Before equations for this calculation can be given, it is advisable to consider the relation of ρ to two other functions, the back pressure and the drop weight.

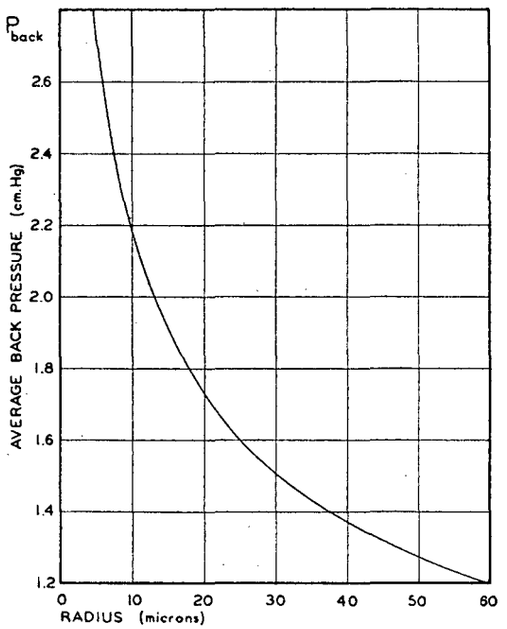

Figure 2. Relation of Average Back Pressure to Radius of Orifice of Dropping Mercury Electrode in 0.1 *N* Potassium Chloride

As has been pointed out (*1*), not all of the "apparent" pressure, P_{app}, existing between tip of capillary and level of mercury in the reservoir is effective in forcing mercury through the capillary. A certain amount of back pressure, P_{back}, which varies with the size of the drop and is equal to P_c when the drop first forms, must be overcome. The effective pressure, P, is found as the difference between the apparent pressure and an average value of the back pressure, or

$$P = P_{app} - \text{average } P_{back} \quad (3)$$

This average back pressure can be expressed as a function of ρ and σ (*1*). In 0.1 *N* potassium chloride, at 25° C., the equation is

$$\text{average } P_{back} = 4.6925\ \rho^{-1/3} \text{ cm.} \quad (4)$$

when ρ is expressed in microns. This relationship is plotted in Figure 2 for easy reference.

The drop weight, W, likewise can be expressed in terms of ρ and σ (*1*). In a solution of 0.1 *N* potassium chloride, at 25° C., one finds the drop weight to be a linear function of ρ:

$$W = 0.24356\ \rho \quad (5)$$

when W is expressed in milligrams and ρ in microns.

The capillary constant, κ, which is equal to P/m, is also equal to Pt/W, since the weight of a drop, W, is equal to mt, where t is the drop time and m the rate of flow of mercury. We can therefore write $Pt = W\kappa$ and, substituting from Equations 1 and 5, get

$$Pt = 5.253 \times 10^5 \times \frac{l}{\rho^3} \quad (6)$$

This leads to the following relationship:

$$\frac{Pt\,\rho^3}{525{,}300} = l \quad (7)$$

which can be used to calculate the length of the capillary; l will be in centimeters, if P is expressed in centimeters, t in seconds, and ρ in microns ($1\ \mu = 10^{-4}$ cm.). To simplify calculations, the nomogram of Figure 3 has been constructed, which represents the above equation.

These equations have been tested on a number of different capillaries. The observed and calculated data for five of these are listed in Table I. The length of the capillaries was measured with

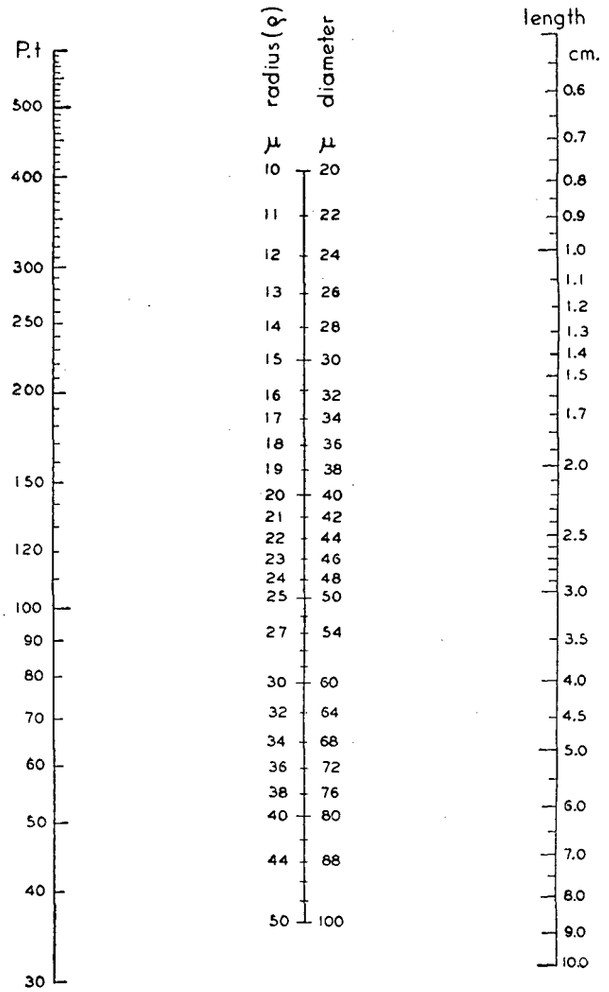

Figure 3. Nomogram

Showing relation of product of effective pressure and drop time, *Pt*, to length and either radius of orifice, ρ, or diameter of capillary

Table I. Comparison of Directly Measured and Calculated Length of Capillaries with Uniform Bore Dropping in 0.1 *N* Potassium Chloride

	Data Observed				Data Calculated				
Capillary	Length, l, cm.	Drop time, t, sec.	Drop weight, W, mg.	Apparent pressure, P_{app}, cm.	Radius of orifice, ρ, μ	Back pressure, P_{back}, cm.	Effective pressure, P, cm.	Pt cm. sec.	Length, l, cm.
C22	8.98	5.91	5.75	61.6	23.6	1.6	60.0	355	8.92
C23	7.03	4.65	5.78	61.6	23.7	1.6	60.0	279	7.07
C24	7.01	8.24	4.76	61.6	19.6	1.7	59.9	494	7.05
C25	3.08	6.28	4.00	61.6	16.4	1.8	59.8	376	3.14
C29	0.80	2.82	3.30	60.0	13.6	2.0	58.0	164	0.79

a rule calibrated in 0.5 mm. The drop time and drop weight values are based on measurements of 20 drops collected in 0.1 N potassium chloride solution. The apparent pressure of mercury was measured as the difference between the tip of the capillary and the level of mercury in the reservoir. The radius of the orifice was calculated by multiplying W by 4.11 (*1*). The back pressure was read off from Figure 2; P is $P_{app} - P_{back}$. The calculated value for the length of the capillary was obtained on the basis of Equation 7. By means of the nomogram (Figure 3) values cannot be read closer than to one decimal unless the length of the capillary is less than 1.5 cm.

It may be seen that there is a satisfactory agreement between directly determined and calculated l values. The two capillaries C22 and C23 were cut from the same piece of marine barometer tubing; their ρ values, which should be identical, illustrate the error of the drop-weight method. Capillary C29 was first sealed to an ordinary piece of glass tubing and then cut to the small length. It is essential that square cuts be made; efforts to grind off a smooth enough surface have so far been to no avail.

On the basis of the above relationships it is possible to determine the exact size of a capillary to perform according to specifications. However, the product actually obtained will depend on the available bore sizes and the accuracy with which the capillary can be cut to size. This may be illustrated by the following example:

A piece of the capillary tubing used for capillaries C22 and C23 was sealed into a regular piece of glass tubing. The seal was made as abrupt as possible, so that there would be a sharp break from the narrow capillary bore to the wider dimension. The capillary was expected to produce a drop time of 2.0 seconds in 0.1 N potassium chloride at an effective pressure of 60 cm., therefore $Pt = 120$. From the nomogram (Figure 3) it was found that the length of the capillary should be slightly greater than 3.0 cm.

After cutting, the capillary length was found to be actually 3.10 cm. In 0.1 N potassium chloride solution the drop time was 2.11 seconds when the applied pressure was 61.6 cm. (effective pressure 60.0 cm.). The small deviation from the intended drop time can easily be overcome by raising the mercury pressure by 3.3 cm.

ACKNOWLEDGMENT

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Acidic Behavior of Concentrated Boric Acid Solutions

DEWITT STETTEN, JR.

Public Health Research Institute of the City of New York, New York, N. Y.

THE use of aqueous boric acid for the quantitative collection and subsequent titration of ammonia appears to have been introduced by Winkler (*17*); there are numerous reports in the literature of the application of this reagent in the quantitative determination of ammonia produced in various analytical procedures. Thus, boric acid is widely employed for the collection of ammonia generated in the Kjeldahl nitrogen method (*1, 4, 6, 9–13, 16*) and in other analytical procedures (*15*). The titration of solutions of ammonia in boric acid with standard hydrochloric acid is usually carried out to a methyl red end point, often in the presence of methylene blue, and by the use of color-matching techniques gives entirely satisfactory results (*7*).

Recently in this laboratory it was noted that after significant quantities of ammonia vapor were delivered into 4% aqueous boric acid containing methyl red–methylene blue indicator, the expected change in color (purple → green) did not occur until after the mixture had been diluted with distilled water. Only after considerable dilution had been effected could the ammonia be satisfactorily titrated with hydrochloric acid. It thus appeared that in concentrated solution boric acid behaved as a stronger acid than in dilute solution and the following experiments were designed to investigate this effect.

EXPERIMENTAL

Materials and Apparatus. Twenty-five grams of recrystallized orthoboric acid were dissolved in water to a final volume of 500 ml.; this solution was employed in subsequent titrations. Sodium hydroxide solution was prepared with carbon dioxide–free water and standardized against sulfamic acid (*3*). The indicator employed contained 1 part of 0.1% methylene blue in 95% ethyl alcohol and 2 parts of 0.1% methyl red in 80% ethyl alcohol. The end point selected was a virtually colorless one lying between the purple (acid) and green (basic) tints.

Measurements of pH were obtained with a Beckman Model H-2 glass electrode instrument and all readings were made at a room temperature of 30° ± 1° C.

Titration of Boric Acid to Indicator End Point. To 20.00-ml. (equivalent to 1.00 gram) aliquots of boric acid solution were added various quantities of distilled water ranging from 0 to 75 ml. After addition of 3 drops of indicator, each sample was titrated with 0.0200 N sodium hydroxide. At the end of titration, the pH of each solution was read on the glass electrode and all were found to lie between 5.2 and 5.4.

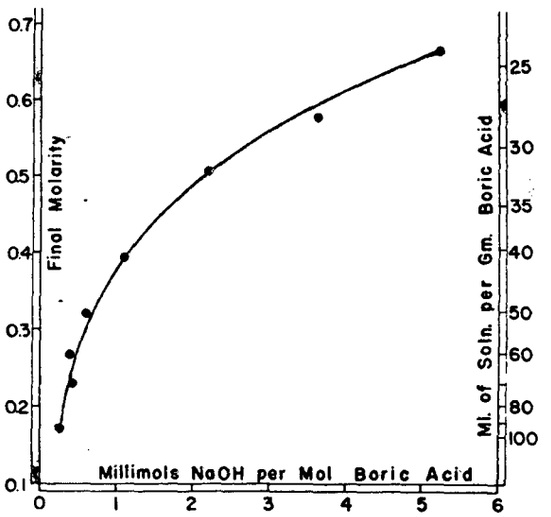

Figure 1. Titration of Boric Acid to Methyl Red End Point

Titration Curves of Boric Acid at Constant Volume. To 20.0-ml. (equivalent to 1 gram) portions of boric acid solution were added quantities of 0.0200 N sodium hydroxide varying from 0 to 5 ml. The final volume was then adjusted by the measured addition of water to a value of 25.0 ml. in one series, and 50.0 ml. in another. The final total concentration of borate plus boric acid was thus 0.647 mole per liter in the first series, 0.323 in the

second. The pH of the contents of each beaker was read on the glass electrode.

Titration of Boric Acid with Water. The pH of aqueous solutions of pure boric acid was read on the glass electrode over a range of concentrations from 0.808 to 0.0311 *M*.

DISCUSSION

It is apparent from the values in Figure 1 that in concentrated solution as much as 0.005 equivalent of alkali may be required to bring boric acid to the turning point of the indicator. This quantity falls off rapidly as the solution is diluted, and virtually disappears when the concentration falls to 0.1 *M* or below. In procedures like the Kjeldahl nitrogen method the recommended quantity of boric acid is in enormous excess, often 50 to 100 fold the amount of ammonia collected, on a molar basis. As usually conducted, the Kjeldahl distillation effects sufficient dilution of the boric acid to minimize the error which would otherwise result. In procedures, however, in which ammonia is delivered into concentrated boric acid by aeration rather than by distillation, proper dilution should precede titration of the ammonia if error is to be avoided. The concentration recommended by Van Slyke *et al.* (*15*), approximately 2% boric acid, would appear to be too high for greatest ease of titration.

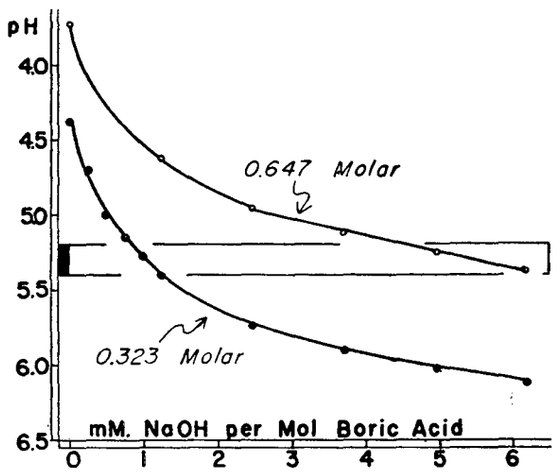

Figure 2. Electrometric Titration Curves of Boric Acid at Two Concentrations

■ Sensitive range of methyl red–methylene blue indicator

The titration of concentrated solutions of boric acid in the presence of methyl red gave indistinct end points, whereas in dilute solution ($M < 0.1$), sharp end points were obtained. The reason for this difference is found in Figure 2, where are plotted the initial segments of titration curves of 1 gram of boric acid conducted at two different volumes. The block in this graph represents the range of turning of the indicator. At the higher concentration about 5 to 6 me. of sodium hydroxide are required to bring the acid into the sensitive range of the indicator whereas halving the concentration reduces this quantity to about 1 me. Furthermore, distinct buffering is evident in the indicator range pH 5.2 to 5.4 when the more concentrated solution is titrated.

The first pK of orthoboric acid is generally given as approximately 9.2 (*2*) and one would not anticipate any extensive buffering effect of a weak acid at pH = pK − 4. The evidence presented thus far fits the hypothesis that in concentrated solutions ($M > 0.1$) of boric acid there exists a molecular species more highly dissociated than orthoboric acid. On the assumption that this new species represents a complex of boric acid with itself, the relationship between pH and concentration of pure boric acid in water has been explored (Figure 3). This relationship in the case of an ideal weak acid is given by the equation,

$$\mathrm{pH} = {}^1/_2\,\mathrm{pK} - {}^1/_2 \log [\mathrm{HA}] \qquad (1)$$

where $K = \dfrac{[\mathrm{H^+}]\,[\mathrm{A^-}]}{[\mathrm{HA}]}$. Line *B*, Figure 3, is the plot of this equation for the situation pK = 9.2. Whereas in dilute solution ($M < 0.05$) the experimental points fall close to this ideal line, at higher concentrations ($M > 0.1$), the points deviate markedly on the side of unexpectedly high acidity, falling close to a straight line (line at left, Figure 3).

In the succeeding analysis advantage is taken of the fact that the ionic strengths of the aqueous solutions of boric acid are low (maximum = $10^{-3.5}$ mole per liter). From the limiting law of Debye and Hückel activity coefficients have been estimated as ranging from 0.98 for the most concentrated to 1.00 for the most dilute solutions of boric acid employed. Consequently it is felt that no important error is introduced by the use of concentrations in lieu of activities in the following equations.

If the reaction, $n\mathrm{HA} \leftrightarrows (\mathrm{HA})_n$, is assumed to occur, where

$$K_1 = \frac{[(\mathrm{HA})_n]}{[\mathrm{HA}]^n} \qquad (2)$$

then

$$[(\mathrm{HA})_n] = K_1\,[\mathrm{HA}]^n \qquad (3)$$

That the abundance of complex is at all times low compared with that of monomeric boric acid is indicated by the failure to detect its occurrence from studies of the colligative properties of boric acid solutions (*5*, *8*). Hence as a first approximation the quantity [HA] in Equation 3 can be taken as equal to the concentration of orthoboric acid introduced. If it further be assumed that only one species of complex exists, that it dissociates as a monobasic acid with an acidic dissociation constant K_2, and

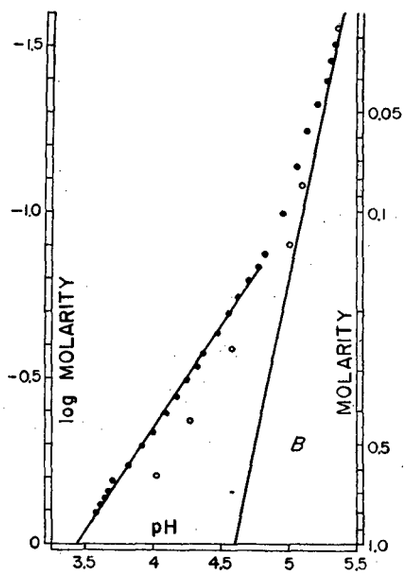

Figure 3. Relationship between Concentration of Boric Acid and pH

● Present experimental points
○ Calculated from data of Thygesen (*14*)

that at high concentrations essentially all the H^+ ions arise from this dissociation of the complex acid, the relationship

$$pH = \tfrac{1}{2}(pK_1 + pK_2) - \frac{n}{2}\log [HA] \quad (4)$$

can be shown to apply.

On the coordinates selected in Figure 3 this also becomes the equation for a straight line, with the following characteristics:

$$\frac{\Delta pH}{\Delta \log [HA]} = -\frac{n}{2} \quad (5)$$

At the intercept, log [HA] = 0.

$$pH = \tfrac{1}{2}(pK_1 + pK_2) \quad (6)$$

For line A these quantities may be evaluated as follows:

$$-\frac{n}{2} = -1.6$$
$$n = 3.2$$
$$\tfrac{1}{2}(pK_1 + pK_2) = 3.43$$
$$pK_1 + pK_2 = 6.86$$

Bearing in mind the assumptions which have been made it may be concluded that the complex is made up, on the average, of 3.2 monomeric molecules and that the product of polymerization and dissociation constants of this complex, $K_1.K_2 = 10^{-6.86}$.

The undue acidity of concentrated boric acid solutions has previously been investigated by Thygesen (*14*) who, on the basis of electrical conductivity measurements, collected data indicating the occurrence in concentrated solution of a complex borate made up of approximately 3 monomeric molecules. Calculations from his data give rise to points included in Figure 3. These points show the same general distribution as those obtained in the present study, with a systematic displacement to the right, doubtless the result of the fact that his measurements were made at a lower temperature, 18° C. Thygesen interprets his data on the assumption of a doubly dissociated tetramer rather than a singly dissociated complex. He further elects to assign dissociation constants to his complex acid in the same ratio as the two dissociation constants of glutaric acid. Inasmuch as both of these judgments appear to be arbitrary to the present writer, it is deemed preferable to describe concentrated solutions of boric acid as behaving as if they contained a far stronger complex acid than orthoboric acid of an average molecular weight 3.2 times that of the monomer.

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Determining Phosphorus in Organic Substances

Micro-Lorenz Method

YOJIRO TSUZUKI AND MAKOTO MIWA, *Tokyo College of Science, Tokyo, Japan*, AND ETSURO KOBAYASHI
Government Chemical Industrial Research Institute, Tokyo-Hatagaya, Japan

A SIMPLE method of determining phosphorus was established by von Lorenz (*2*) by weighing directly the ammonium phosphomolybdate which was precipitated with the molybdate reagent containing ammonium sulfate. This method was adopted by Hans Lieb for the microdetermination of organic phosphorus, as described in Pregl's book (*3*). Although precipitates of the same composition are always obtained under definite conditions, a factor for phosphorus must be empirically determined, because its composition is not exactly known. Lieb used secondary ammonium phosphate, $(NH_4)NaHPO_4.4H_2O$, for the material of standard analysis, and found, as the result of six analyses, a value of 0.03326 as the phosphorus pentoxide content of the yellow precipitate, corresponding to 0.014524 as the phosphorus factor. There are, however, errors in the original table given in Pregl's book. These factors, when corrected and revised referring to the latest atomic weights, become somewhat larger than before—that is, 0.033315 and 0.01454, respectively. In this paper a new factor for phosphorus is reported, because the former factors are a little too large.

The authors have used primary potassium phosphate, KH_2PO_4, analytical reagent grade, as a material of standard analysis. This anhydrous salt was purified by repeated crystallization from water and dried in an air bath at 110° C. for 3 hours; it lost no appreciable weight when heated at 110° C. in vacuo for a few hours and scarcely gained in weight when preserved in a desiccator or in a well-stoppered bottle. The salt, when analyzed by the Schmitz method, contained the theoretical quantity of phosphorus.

The phosphate, 0.2247 and 0.3027 gram, gave respectively, 0.1837 and 0.2475 gram of magnesium pyrophosphate. The phosphorus found was 22.75%. Calculated for monopotassium phosphate, phosphorus equals 22.76%.

The results of a series of analyses using this pure anhydrous salt are shown in Table I.

As may be seen from Table I, the content of phosphorus in the

Table I. Phosphorus Content of Ammonium Phosphomolybdate

Quantity of KH_2PO_4, Mg.	Corresponding Quantity of Phosphorus, Mg.	Ammonium Phosphomolybdate Obtained, Mg.	Phosphorus in the Precipitate, %
1.724	0.3924	27.27	1.439
2.218	0.5048	35.04	1.441
2.612	0.5945	41.15	1.445
3.883	0.8838	61.35	1.441
3.919	0.8920	61.79	1.444
5.805	1.3212	91.65	1.442
6.704	1.5258	105.88	1.441
7.095	1.6148	111.78	1.445

precipitate is nearly constant. The arithmetical mean of the eight figures of the last column is 1.442 and thus 0.01442 may be taken as a phosphorus factor; correspondingly, 0.03304 may be taken as a phosphorus pentoxide factor. This value is somewhat smaller than Pregl's factor, and approaches the original value of 0.03295 obtained by N. von Lorenz in establishing the macromethod.

Table II. Time Necessary for Drying Halogen Filter Tube

Weight of Halogen Filter Tube, Grams	Time of Drying, Min.	Weight of Halogen Filter Tube with Precipitate, Grams	Time of Drying, Min.
2.01214[a]	4	2.08431	3
2.01210	10	2.08416	12
2.01209	37	2.08411	20
2.01488[a]	10	2.08411	25
2.01487	30	2.08412	63

[a] Of different series.

The analyses were carried out as follows:

The sample of monopotassium phosphate is weighed and dissolved in dilute nitric acid, and 2 ml. of the nitric acid containing sulfuric acid are added. The solution is made up to a volume of 15 ml. with water and heated at 80° C. in a water bath, and 15 ml. of the sulfate-molybdate reagent are run into it from a pipet as rapidly as possible. The solution is allowed to stand for 4 to 5 minutes, after which the greater part of the precipitate settles down. The vessel is then shaken half a minute and allowed to stand for 2 to 5 hours. Then the precipitate is filtered in a weighed halogen filter tube, washed with the 2% ammonium nitrate solution, and dried with alcohol and acetone. The filter with the precipitate is placed for 20 minutes in a desiccator evacuated to a pressure of 25 to 60 mm., and it is weighed 3 minutes after removal from the desiccator. Thus the constancy of the weight of the filter tube either alone or with the precipitate can easily be attained.

Because absolutely pure preparations of organophosphorus compounds were not at the authors' disposal, the phosphorus contents of casein and tricresyl phosphate have been accurately determined by the method of Schmitz. The results of the microanalysis using the new factor agree fairly well with the analyses by the Schmitz method, whereas Lieb's factor gives considerably higher values.

Casein. (Phosphorus equals 0.738 ± 0.001% by the Schmitz method.) Casein, 0.1003 and 0.1068 gram, yielded 51.173 and 54.936 mg. of ammonium phosphomolybdate, respectively. The phosphorus found was 0.736 and 0.741%, respectively.

Tricresyl Phosphate. (Phosphorus equals 8.365 ± 0.000% by the Schmitz method.) A 1.872-mg. portion yielded 108.78 mg. of ammonium phosphomolybdate. The phosphorus found was 8.379%.

It is not desirable to shake the vessel until the greater part of the precipitate has settled down. Usually it takes more than 3 minutes.

Pregl states that the precipitate should be allowed to stand at least 1 hour, but it is likely that 1 hour is not sufficient inasmuch as the precipitation is often incomplete after 1 hour. Thus, the vessel must be allowed to stand for at least 2 hours. When the precipitation is not complete, the supernatant liquid is faintly yellow. From a faintly colored filtrate a further precipitate will form on standing. The total sum of these precipitates is almost equal to the value expected. This is illustrated by the following experiment.

A 2.003-mg. portion of monopotassium phosphate gave 30.08 mg. of the yellow precipitate. Although the precipitate was filtered after the vessel was allowed to stand for 2 hours, the precipitation was incomplete and the filtrate was pale yellow, probably because the vessel was shaken too early. From the colored filtrate, 1.45 mg. of precipitate were obtained after a day. The total sum of the precipitate was 31.53 mg. in weight; the content of phosphorus in the precipitate was calculated as 0.01445.

The time necessary for drying the filter in a vacuum desiccator is shorter than that given by Pregl (30 minutes), as shown by Table II.

The authors have investigated the effect of acid quantity on the yellow precipitate, because this effect has often been noticed (*1*), and because the acid quantity of the medium from which the precipitate is formed is variable, owing to the relative difficulty of conversion of organic phosphorus into phosphoric acid.

The studies were carried out as follows: the yellow precipitate is formed by the method described herein from solutions of monopotassium phosphate containing various quantities of the nitric–sulfuric acid. The results obtained are recorded in Table III, where the solution of monopotassium phosphate used as a material of analysis contains 1.4915 grams of the salt in 1 liter.

Table III. Effect of Acid Quantity on Precipitation

KH_2PO_4, Ml.	HNO_3–H_2SO_4, Ml.	Yellow Precipitate, Mg.	$P_{calcd.}$, Mg.	$P_{obsd.}$, Mg.[a]	Error, %
2	5	47.16	0.679	0.679	0.0
2	6	47.19	...	0.679	0.0
2	10	47.17	...	0.679	0.0
3	2	70.47	1.018	1.014	0.4
3	6	70.99	...	1.022	+0.4
3	10	70.78	...	1.019	+0.1
5	2	117.72	1.697	1.694	−0.2
5	10	117.66	...	1.693	−0.2

[a] Calculated with the new factor, 0.01442.

The data in Table III lead us to conclude that the acid does not exercise any systematic effect on the precipitation, when between 2 and 10 ml.

The effect of the quantity of the molybdate reagent on the precipitation has also been studied in a similar way, except that the yellow precipitate was determined volumetrically by neutralizing it with 0.1 *N* sodium hydroxide. The results are given in Table IV; each of the figures in the third column is the mean of three values obtained with three solutions containing, respectively, 2, 5 and 10 ml. of the nitric-sulfuric acid.

Table IV. Effect of Quantity of Molybdate Reagent on Yellow Precipitate

KH_2PO_4, Ml.	Molybdate Reagent, Ml.	0.1 *N* NaOH, Ml.
3	7.5	9.32
3	10	9.47
3	15	9.42
4	7.5	12.51
4	10	12.56
4	15	12.57
5	7.5	15.21
5	10	15.93
5	15	15.92

Table IV shows that when the molybdate reagent is between 10 and 15 ml. in volume it is indifferent to the quantity of the precipitate, but when the volume is less than 10 ml., it gives constantly less precipitate.

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Determination of Mercury and Bromides with 1,10-Phenanthroline Ferrous Sulfate

M. E. HALL[1] WITH GEO. M. SMITH
Vanderbilt University, Nashville, Tenn.

THE 1,10-phenanthroline ferrous complex, as the sulfate, was prepared and described by Blau (*1*) in 1898. Walden, Hammett, and Chapman (*12*) and others (*10, 11, 13–16*) described its use as a high potential, reversible oxidimetric indicator. The formation of the highly colored complex has been used for the colorimetric determination of iron (*3, 6–8*).

Blau (*1*) noted that the complex, called ferroin by Gleu (*4*), formed insoluble salts with chloroplatinate and picrate. Smith (*9*) prepared insoluble perchlorates and periodates. Feigl and Miranda (*2*) used ferroin to detect cadmium by forming a precipitate with CdI_4^{--}, and noted also that HgI_4^{--} and BiI_4^- formed precipitates with ferroin. These properties suggested its use in the quantitative determination of mercury.

GRAVIMETRIC DETERMINATION OF MERCURY

Reagents. Ferroin, 0.025 *M* solution of 1,10-phenanthroline ferrous sulfate, supplied by the G. Frederick Smith Chemical Co., Columbus, Ohio.

Potassium bromide, 2% aqueous solution.

Mercuric nitrate, crystals, free from halide ions. Solutions were standardized against standard potassium thiocyanate solution, using saturated ferric alum solution as the indicator. Potassium thiocyanate was standardized against standard silver nitrate solution, using ferric alum as the indicator.

The cations investigated for interference were added as nitrates. The anions were added as sodium or potassium salts.

Principles. The chemistry of this method, and of the volumetric methods for mercury and bromide, is stated in the following equations:

$$Hg^{++} + 2Br^- \longrightarrow HgBr_2 \text{ (very weakly dissociated)} \quad (1)$$

$$HgBr_2 + Br^- \longrightarrow HgBr_3^- \quad (2)$$

$$2HgBr_3^- + (C_{12}H_8N_2)_3Fe^{++} \longrightarrow (C_{12}H_8N_2)_3Fe(HgBr_3)_2 \quad (3)$$

The product of Reaction 3 is an insoluble red compound, ferroin tribromomercuriate, which can be filtered, washed, dried, and weighed as such.

Composition of Precipitate. The composition of the precipitate, the product of Equation 3, was established by determining the quantity of mercuric ion required to precipitate a known quantity of ferroin, by weighing the precipitate formed by a known quantity of mercuric ion, and by determining iron and bromine in the precipitate.

MERCURY REQUIRED TO PRECIPITATE KNOWN QUANTITY OF FERROIN. Standard mercuric nitrate was added, a little at a time, to a known quantity of ferroin, containing an excess of bromide, until a colorless filtrate could be obtained. Thus, 7.00 ml. of 0.05 *M* mercuric nitrate were required to react with 7.00 ml. of 0.025 *M* ferroin. As the ratio of gram-atoms of mercury to moles of ferroin is therefore 2 to 1, the precipitate is ferroin tribromomercuriate $[(C_{12}H_8N_2)_3Fe(HgBr_3)_2]$.

WEIGHT OF PRECIPITATE FORMED BY KNOWN QUANTITY OF MERCURY. Known quantities of mercuric ion, containing an excess of bromide, were precipitated completely with an excess of ferroin. Thus, 16.5 mg. of mercuric ion formed a precipitate weighing 61.2 mg. (27.0% mercury); 66.0 mg. formed a precipitate weighing 246.5 mg. (26.8% mercury). The theoretical percentage of mercury in ferroin tribromomercuriate is 27.2, of ferroin tetrabromomercuriate is 18.0% mercury.

ANALYSIS OF PRECIPITATE. A weighed precipitate was oxidized by nitric acid, then ignited to volatilize mercury (*5*). The residue was weighed as ferric oxide. The iron content was 3.73% as compared with a theoretical 3.78% in $(C_{12}H_8N_2)_3Fe(HgBr_3)_2$ and 5.00% in $(C_{12}H_8N_2)_3FeHgBr_4$.

Bromide was determined by treating a nitric acid suspension of the precipitate with silver nitrate, and weighing the silver bromide formed. Thus, bromine found was 32.34% as compared with the theoretical 32.46% in $(C_{12}H_8N_2)_3Fe(HgBr_3)_2$ and 28.64% in $(C_{12}H_8N_2)_3FeHgBr_4$.

[1] Present address, Humble Oil Co., Baytown, Tex.

Physical Properties of Precipitate. Ferroin tribromomercuriate is a dark red crystalline substance, sparingly soluble in water, insoluble in potassium bromide solution, hydrobromic acid solution, diethyl ether, methyl acetate, and ethyl acetate, but soluble in ethyl alcohol and acetone. It can be heated to 275° C. without decomposition, and dries to constant weight at 110° C. The formula weight is 1476.6; the gravimetric factor for mercury is 0.2726.

The precipitate is somewhat soluble in water at 25° C. (3.6 mg. per 100 ml. of water) and sparingly soluble (0.5 mg. per 100 ml. of water) at 5° C. Because only 0.1 mg. dissolves in 100 ml. of 0.002 *M* hydrobromic acid, all precipitates were washed with this solution; 250 mg. of precipitate, washed with 0.002 *M* hydrobromic acid, dries to constant weight in 1 hour at 110° C. If this washing is followed by methyl acetate and ether, the precipitate dries completely in 10 to 15 minutes.

Effect of Bromide Ion Concentration. Quantitative precipitation occurs in concentrations of excess bromide 0.002 *M* or greater. If excess bromide exceeds 0.03 *M*, results are always high, probably because some $(C_{12}H_8N_2)_3FeHgBr_4$ forms. Ferroin bromide does not precipitate in bromide concentrations as great as 2.0 *M*. Quantitative results cannot be obtained in high bromide concentrations, as the tetrabromo compound apparently is not quantitatively insoluble. Furthermore, in high bromide concentrations, cadmium interference is greater because of the precipitation of its bromide complex.

Effect of Excess Ferroin. In bromide concentrations as great as 0.002 *M*, complete precipitation of ferroin tribromomercuriate occurs if the concentration of excess ferroin is at least 0.0001 *M*. Larger excesses are not objectionable, but are not needed.

Quantities of Mercury Determined. Quantities of mercury from 1.00 to 66.00 mg. have been determined gravimetrically (Table I).

Table I. Mercury Determination.

Mercury Present, Mg.	Mercury Found, Mg.
1.00	0.98
5.00	4.97
10.00	10.10
16.50	16.70
20.00	19.90
66.00	66.90

For smaller quantities the solubility error is too large, and the difficulty of washing heavier precipitates prevents the range of determinations from being extended.

Procedure. A weakly acid solution of 1 to 60 mg. of mercury, as mercuric nitrate, is mixed with 2% potassium bromide solution to give a bromide concentration 0.002 to 0.02 *M* in 100 ml. The pH of the solution should be about 5. After dilution to about 100 ml. with water, 2 to 7.5 ml. of 0.025 *M* ferroin are added until no no additional precipitate forms. The suspension is stirred, allowed to stand 10 to 15 minutes, and filtered through a Gooch or filtering crucible. The precipitate is washed with 0.002 *M* hydrobromic acid solution, dried for an hour at 110° C., cooled, and weighed.

Effect of Added Substances. The effects of free nitric, sulfuric, hydrochloric, and acetic acids on quantitative precipitation were studied by attempting precipitation in 0.1 to 2.0 *M* concentrations. Acetic acid, up to 2.0 *M*, causes no interference, but the other acids interfere seriously. Solubility is greatest in hydrochloric acid, probably because of the stability of $HgCl_3^-$ or $HgCl_4^{--}$. Solubility in sulfuric acid possibly is due to mercury

Table II. Determination of Mercury in Presence of Other Substances

(Hg present 10.00 mg. 0.0001 *M* Xs ferroin Vol. 100 ml.)

Substance Added	Quantity Added, *M*	Bromide Concentration, *M*	Mercury Found, Mg.
$HC_2H_3O_2$	2.0	0.0033	10.01
HNO_3	1.0	0.0033	9.70
HNO_3	2.0	0.0033	9.40
H_2SO_4	0.05	0.0033	9.85
H_2SO_4	0.5	0.0033	9.50
HCl	0.05	0.0033	9.85
HCl	0.12	0.0033	9.30
NH_4^+[a]	1.5	0.002	10.00
Na^+	2.0	0.002	10.20
	Mg.		
Pb^{++}	100	0.002	10.00
Cu^{++}	100	0.002	10.10
Cd^{++}	100	0.002	10.00
Fe^{+++}	100	0.002	10.00
Cr^{+++}	100	0.002	10.00
Ni^{++}	100	0.002	10.00
Mn^{++}	100	0.002	10.10
Al^{+++}	100	0.002	10.00
Zn^{++}	100	0.002	10.10
Ca^{++}	100	0.002	10.10
Mg^{++}	100	0.002	9.90
	M		
Cl^-	0.005	0.002	10.00
Cl^-	0.025	0.002	9.85
Cl^-	0.5	0.002	8.65
Cl^-	0.2	0.03	10.00
Cl^-	0.5	0.03	9.10
SO_4^{--}	0.4	0.02	9.85
SO_4^{--}	0.5	0.003	10.20
NO_3^-	2.0	0.002	10.10
$C_2H_3O_2^-$	2.0	0.002	10.00

[a] $NH_4C_2H_3O_2$ added

sulfate complexes, and to a change of ferroin to 1,10-phenanthroline. Interference by free nitric acid probably is caused by partial conversion of ferroin to ferriin (the oxidized form of the indicator) and to 1,10-phenanthroline (Table II).

Various cations and anions were added to known quantities of mercuric ion and precipitation was attempted as usual (Table II). High ammonium concentrations delay precipitation, but good results can be obtained by allowing a longer time for precipitation and using more bromide. Moderate concentrations of lead do not interfere if ammonium acetate is added to prevent precipitation of lead sulfate and lead bromide. Sulfate ion concentration is too low to precipitate calcium sulfate. If acidity is increased to prevent hydrolysis of bismuth(III), mercury is not removed completely. If bromide concentration is increased, ferroin precipitates the bismuth bromide complex. Silver interferes by forming a precipitate of silver bromide.

Chloride interferes by forming $HgCl_3^-$ and $HgCl_4^{--}$ which do not precipitate quantitatively with ferroin. Sulfate ion, up to 0.05 *M*, has slight interference, which may be overcome considerably by increasing bromide concentration. Nitrate, at pH 5, does not interfere unless present in concentrations of more than 2.0 *M*. Thiocyanate ion forms mercury complexes which precipitate with ferroin, and forms ferroin thiocyanate. Iodide interferes by forming iodomercury complexes which precipitate with ferroin, insoluble mercuric iodide, iodo complexes with other cations, such as cadmium, which precipitate with ferroin, and insoluble ferroin iodide in iodide concentrations greater than 0.4 *M*.

CHLOROMERCURY AND IODOMERCURY COMPOUNDS WITH FERROIN

The possibility of determining mercury as ferroin chloromercury salt was investigated with satisfactory results, because the salts are too soluble. Only partial precipitation occurs in 30 minutes in 1 *M* chloride. At 25° C., 40 mg. of the precipitate dissolve in 100 ml. of water.

Mercury seems to be precipitated quantitatively as the iodo-complex, but the composition is uncertain, corresponding approximately to an equimolar mixture of the triiodo and tetraiodo-complexes. If the iodomercury solutions are heated almost to boiling, and then cooled before the addition of ferroin, the precipitate seems to be altogether ferroin tetraiodomercuriate. Nevertheless, the precipitation of the iodo complex is unsatisfactory. The precipitate does not filter and wash well.

VOLUMETRIC DETERMINATION OF MERCURY

Principles. The chemistry of this method is stated in Equations 1, 2, and 3. Reaction 2 does not occur until mercury has been converted quantitatively into the very weakly dissociated mercuric bromide by Reaction 1. The first drop of bromide solution in excess of that required for Reaction 1 produces $HgBr_3^-$, which immediately reacts with ferroin to form a precipitate, as stated by Equation 3. Therefore, the equivalence point, denoted by the formation of a very small amount of reddish precipitate, is reached when 2 gram-ions of bromide have been added for each gram-ion of mercury(II).

Procedure. Potassium bromide solution, standardized by precipitation as silver bromide, is run into mercuric nitrate solution, standardized as stated above. The solution contains 1 to 4 drops of ferroin as the indicator. The end point is the formation of a permanent pink turbidity, and is sharp and accurate. Reaction (3), producing the end point, is reversible, so that if too much potassium bromide is added, an excess of standard mercuric nitrate may be added and the titration continued with potassium bromide. For small quantities of mercury the end point is somewhat indistinct unless at least 3 drops of indicator are used. This is satisfactory for all titrations, but less indicator may be used with large amounts of mercury.

Table III shows that 10 to 500 mg. of mercury can be determined by this method, in a volume of about 100 ml.

Effect of Added Substances. Free acetic acid, in concentrations exceeding 0.05 *M*, interferes significantly, possibly because of the formation of weakly dissociated mercuric acetate. This is contrary to the effect noted in the gravimetric determination, possibly because mercuric acetate changes into $HgBr_3^-$ on standing. Free sulfuric acid has negligible effect, even when 1 *M*, because conversion of ferroin to 1,10-phenanthroline is not rapid enough to destroy the indicator. The effect of nitric acid is somewhat greater, but not considerable, for the same reason, and be-

Table III. Mercury Determination

Mercury Present, Mg.	Mercury Found, Mg.
504.2	503.8
302.6	302.6
220.0	219.6
201.7	201.9
110.0	110.2
55.00	54.90
50.42	50.50
25.21	25.16
10.10	10.20

Table IV. Effect of Added Substances

(228.0 mg. of Hg present. Ferroin 3 drops. Volume 100 to 125 ml.)

Substance Added	Amount Added, *M*	Mercury Found, Mg.
HNO_3	1.0	228.7
H_2SO_4	1.0	227.6
$HC_2H_3O_2$	0.05	227.6
$HC_2H_3O_2$	0.1	226.5
$HC_2H_3O_2$	0.5	225.4
	Mg.	
Cl^-	10.0	200.3
Cl^-	1.0	224.3
SO_4^{--}	1000	227.5
MoO_3	400	229.4 in 0.6 *M* HNO_3
	M	
Na^+	1.0	228.3
NH_4^+	0.2	227.7
NH_4^+	0.5	227.1
NH_4^+	1.0	226.0
	Mg.	
Bi^{+++}	500	227.6 in 0.3 *M* HNO_3

cause its oxidation of ferroin to ferriin is slow. Chloride, in neutral or acid solution, cannot be tolerated.

As in the gravimetric procedure, most of the common cations do not interfere. The slight effect of ammonium may be overcome by titrating slowly near the end point. Silver interferes by precipitating silver bromide.

Molybdate, tungstate, and metavanadate ions interfere in both slightly acid and slightly alkaline solutions. In acid solutions, mercuric salts of these anions precipitate, while on the alkaline side their ferroin salts precipitate. Vanadate and molybdate do not interfere seriously in 0.5 *M* nitric acid solution. Vanadyl ion does not interfere.

The more common cations (500 mg.) had no noticeable effect on the titration of 228.0 mg. of mercury. The effect of added substances is indicated in Table IV.

Table V. Bromide Determination

Bromide Present, Mg.	Bromide Found, Mg.
271.0	270.7
135.5	135.1
27.10	26.91
13.55	13.35
9.03	9.98
4.52	4.53

VOLUMETRIC DETERMINATION OF BROMIDE

Bromide may be determined by titration with standard mercuric nitrate solution, using 3 drops of ferroin as indicator. The procedure is similar to that used in the titration of mercury, but standard mercuric nitrate is run into the bromide solution. The red precipitate forms with the first few drops of mercuric nitrate and disappears at the end point. This procedure, however, is not so satisfactory as that in which an excess of standard mercuric nitrate is added and the excess titrated by standard potassium bromide to the appearance of a faint pink turbidity. This end point is more distinct than that which depends upon the disappearance of the precipitate. This determination is subject to the same interferences as the determination of mercury. Chloride and iodide are particularly objectionable. Table V indicates results obtained for bromide.

CONCLUSIONS

The sulfate solution of 1,10-phenanthroline ferrous complex (ferroin) can be used to determine 1 to 66 mg. of mercury by precipitation of the red compound, ferroin tribromomercuriate, from solutions containing bromide ion 0.002 to 0.03 *M* in excess. The precipitate can be washed with 0.002 *M* potassium bromide solution and dried in 1 hour at 110° C. The composition of the precipitate is given by the formula $(C_{12}H_8N_2)_3Fe(HgBr_3)_2$; the gravimetric factor for mercury is 0.02726.

The method is most satisfactory at pH 5, but considerable acetic acid does not interfere. Chloride and iodide ions are particularly objectionable. Most of the common cations, except bismuth and silver, do not interfere.

Ferroin can be used as an indicator in the volumetric determination of mercuric and bromide ions. Mercuric ion is titrated directly by standard potassium bromide to the appearance of a faint pink turbidity. Bromide ion is best titrated by adding an excess of standard mercuric nitrate and back-titrating with standard potassium bromide. In either case the end point is reached when 2 gram-ions of bromide have been added for each gram-ion of mercuric ion; 10 to 500 mg. of mercuric ion and 5 to 270 mg. of bromide ion can be determined. Chloride and iodide ions interfere.

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Zirconium for Sodium Peroxide Fusions

G. J. PETRETIC

U. S. Atomic Energy Commission, New Brunswick Laboratory, New Brunswick, N. J.

SODIUM peroxide is a powerful disintegrator and a thorough an defficient flux for the decomposition of ferrosilicon, chrome ore, and other difficultly fusible material. These fusions are generally carried out in nickel or iron crucibles, heated in an electric muffle at 600° to 700° C. or over a free flame. The crucibles are appreciably attacked by the sodium peroxide and large amounts of metal from them are introduced into the melt. Repeated fusions in the same crucible are not recommended because of possible loss of sample.

To overcome the excessive attack on the iron or nickel crucible in the peroxide fusion, a modification of the well-known sodium peroxide explosion method for sulfur in coal and coke has been used (*1*). The proper amount of sugar carbon is mixed with the sample and sodium peroxide in a nickel crucible and the contents are ignited. The crucible is immersed in water during the ignition and subsequent cooling. The disadvantages of this method are that one and a half to two times as much sodium peroxide is used as when fusing over an open flame, and a small amount of unfused residue may remain which, in the case of ferrosilicon and chrome ore, amounts to from 20 to 30 mg. for 1-gram sample.

Recently work has been directed toward the low temperature decomposition of ores by sodium peroxide in platinum (*2–4*). Rafter and Seelye found that at 480° ± 20° C. complete decomposition of zircon results. They discussed the mechanism of the peroxide decomposition (*2*).

At the Atomic Energy Commission Laboratory in New Brunswick, on the other hand, sodium peroxide fusion of ores over an open flame has been successfully carried out in zirconium crucibles. Fusions are quickly made, no temperature control has been necessary, and zirconium crucible is only slightly attacked. Fusions have been carried out over twenty times in the same

Table I. Effect of Molten Sodium Peroxide on Bureau of Mines Zirconium

(50 grams of Na_2O_2 in 168-gram Zr dish heated over open flame)

Treatment	Loss of Weight of Dish			Chemical[a] Analysis
	Mg.	*%*	*Mg./g. Na_2O_2*	*Mg. Zr/g. Na_2O_2*
1. Na_2O_2 heated 15 minutes until molten and then 2 minutes more	188	0.11	3.8	2.6
2. 1 repeated with 50 grams more of Na_2O_2	217	0.13	4.3	2.1
3. 2 repeated	205	0.12	4.1	2.9
4. 2 repeated but heated for about 25 minutes	360	0.22	7.2	5.7

[a] Sodium peroxide melt dissolved in water and HCl. Zr precipitated with NH_4OH, filtered, ignited, and weighed as ZrO_2.

crucible without showing an appreciable attack. A few tests have shown a crucible loss of about 5 mg. for each gram of sodium peroxide, whereas fusions carried out similarly in nickel, iron, and silver lost twenty to fifty times as much. Moreover, as both nickel and iron were to be determined, the use of zirconium for the fusions was ideal, giving no interferences.

The zirconium crucibles were machined from both the de Boer process zirconium bar and the Bureau of Mines vacuum-cast metal. The high purity de Boer metal is more corrosion-resistant and is less attacked during the fusions. The crucibles are approximately 1.375 inches long, $^{15}/_{16}$ inch in diameter at the top, tapering to $^{13}/_{16}$ inch at the bottom, and $^{3}/_{64}$ inch thick. A 3-inch diameter, round-bottomed $^{3}/_{64}$ inch thick zirconium dish weighing about 168 grams was also machined from the Bureau of Mines metal. However, because zirconium is converted to the oxide at elevated temperature, the bottom of the dish, which is directly subject to the open flame, was made 0.25 inch thick.

In Table I are given the results obtained by fusing 50-gram amounts of sodium peroxide in the zirconium dish. The melts after cooling were easily removed by inverting the dish and striking it sharply against a flat surface. The dish was washed with concentrated hydrochloric acid before each weighing.

The dish after each fusion showed a white film of zirconium oxide on the inside where the molten sodium peroxide came in contact with the dish and on the bottom where the direct flame of the burner was applied. If the fusion were performed in a muffle at the lowest temperature necessary for fusion, and if the more corrosion-resistant de Boer process zirconium were used, the losses would be appreciably lessened. The disadvantage of the de Boer type metal is its expense, more than ten times the cost of the vacuum-cast metal. The studies conducted by Seelye and Rafter (*2*, *3*) in platinum should also be of value in determining the optimum condition for fusions in zirconium.

Zirconium metal is rapidly attacked by hydrofluoric acid, and appreciably by phosphoric and sulfuric acids. Nitric and hydrochloric acids have little effect.

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Analysis of Quaternary Mixtures

Application of a Graphical Solution of Four Simultaneous Linear Equations

JAMAL TADAYON

The Metal Box Co., Ltd., Acton, London W.3, England

IN QUANTITATIVE analysis of quaternary mixtures it is usual to determine four independent physical properties for each of the pure components and the corresponding properties for the mixture; then if the linear mixture law holds

$$a_1x + a_2y + a_3z + a_4w = A \quad (1)$$

$$b_1x + b_2y + b_3z + b_4w = B \quad (2)$$

$$c_1x + c_2y + c_3z + c_4w = C \quad (3)$$

$$d_1x + d_2y + d_3z + d_4w = D \quad (4)$$

$$x + y + z + w = 1 \quad (5)$$

where A, B, C, and D are the measured values of the four physical properties for the mixture, and a_i, b_i, c_i, and d_i (i = 1, 2, 3, and 4) are the corresponding values for the four pure components X, Y, Z, and W, whose fractional concentrations in the mixture are x, y, z, and w, respectively. Equation 5 is normally used for checking the results of the analysis obtained from the simultaneous Equations 1, 2, 3, and 4. It is equally possible to solve four equations composed of Equation 5 and any three of the four Equations 1, 2, 3, and 4. The remaining equation is then used for checking purposes. Whichever procedure is adopted, the problem is one of solving four simultaneous linear equations. The speed and the degree of accuracy evidently determine the method of solution, which is usually carried out by successive elimination of the variables or by the use of determinants. As an alternative to these methods, the following simple graphical method has been developed. Its accuracy, as with all other graphical methods of solving mathematical problems (*1*) or other graphical aids to computation, depends on the scale used and the care with which the lines are drawn and measured.

PRINCIPLE OF METHOD AND PROCEDURE

A quaternary system expressed by Equations 1, 2, 3, and 5 may be represented geometrically as a pyramid shown in Figure 1, where points X, Y, Z, and W represent the four pure components in space. Each point is defined by plotting the coefficients a_i, b_i, and c_i for the particular pure component and therefore can be regarded as fixed with respect to the orthogonal axes, a, b, and c. If the mixture law holds, a point representing a binary mixture of X and Y will plot on line XY, that representing a mixture of Y and Z will lie on line YZ, and so on. Similarly, a point

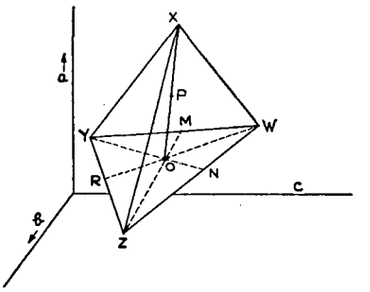

Figure 1. Geometrical Representation of a Quaternary Mixture

representing a ternary mixture of, say, X, Y, and Z will lie inside triangle XYZ. Finally, a point such as P, representing a quaternary mixture of X, Y, Z, and W, will fall in the space bound by the four surfaces of the pyramid. If line XP intersects the plane of triangle YWZ at O, the fractional concentration of X will be given by

$$x = OP/OX \tag{6}$$

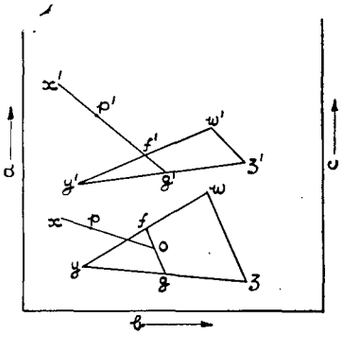

Figure 2. Graphical Solution of Four Linear Simultaneous Equations

Point O can now be considered to represent a ternary mixture of Y, Z, and W, the fractional concentration of each of which may be given by

$$y = (NO/NY)(1 - x) \tag{7}$$

$$z = (MO/MZ)(1 - x) \tag{8}$$

$$w = (RO/RW)(1 - x) \tag{9}$$

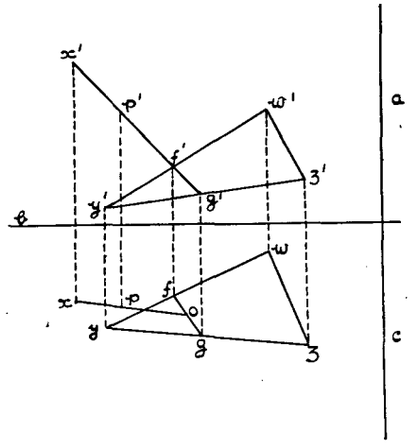

Figure 3. Vertical and Horizontal Projections of Pyramid Representing a Quaternary Mixture

The graphical procedure, the proof of which is given below, is as follows:

In Figure 2 let x', y', z', and w' represent plots of a_i against b_i (i = 1, 2, 3, and 4) in Equations 1 and 2; and x, y, z, and w represent plots of c_i against b_i in Equations 3 and 2 for each of the four pure components, respectively. Similarly, by plotting A and C against B from Equations 1, 3, and 2 we obtain points p' and p, respectively, for the quaternary mixture, P.

Let $x'p'$ intersect any two sides of triangle $y'w'z'$, such as $y'w'$ and $y'z'$, at f' and g'. Produce $f'f$ and $g'g$ at right angles to axis b and draw fg. (Points f and g are easily obtained by inspection if the figure is drawn on graph paper.) Produce xp to intersect fg at O. The fractional concentration of X is then given by $x = op/ox$. Point o is now regarded as representing a ternary mixture of Y, Z, and W and the solution is carried out as described and given by the expressions 7, 8, and 9.

Because coefficients a_i, b_i, and c_i are constants characteristic of the pure components, points x', y', z', w', x, y, z, and w can be plotted permanently on a convenient and constant scale and used for the solution of any mixture composed of X, Y, Z, and W. The points that characterize a particular mixture are p' and p and only these are freshly plotted.

When x, y, z, and w are not given as fractions—i.e., when Equation 5 is not valid—the following transformation of Equation 4 may be carried out.

$$\frac{d_1}{D}x + \frac{d_2}{D}y + \frac{d_3}{D}z + \frac{d_4}{D}w = 1 \tag{10}$$

Putting $X = d_1x/D$, $Y = d_2y/D$, $Z = d_3z/D$, and $W = d_4w/D$, and substituting, results in:

$$\frac{a_1}{d_1}X + \frac{a_2}{d_2}Y + \frac{a_3}{d_3}Z + \frac{a_4}{d_4}W = A/D \tag{11}$$

$$\frac{b_1}{d_1}X + \frac{b_2}{d_2}Y + \frac{b_3}{d_3}Z + \frac{b_4}{d_4}W = B/D \tag{12}$$

$$\frac{c_1}{d_1}X + \frac{c_2}{d_2}Y + \frac{c_3}{d_3}Z + \frac{c_4}{d_4}W = C/D \tag{13}$$

$$X + Y + Z + W = 1 \tag{14}$$

The equations are now in a convenient form for graphical solution. The values of x, y, z, and w can be obtained from X, Y, Z, and W, respectively.

PROOF

The fractional concentration of X can be given by OP/OX (Figure 1). As points X and P are defined, it remains to locate point O at which XP intersects the base, YZW.

Project the pyramid on the vertical plane containing axes a and b and on the horizontal plane containing axes b and c to obtain Figure 3, where the vertical plane is rotated anticlockwise through 90° to lie in the horizontal plane.

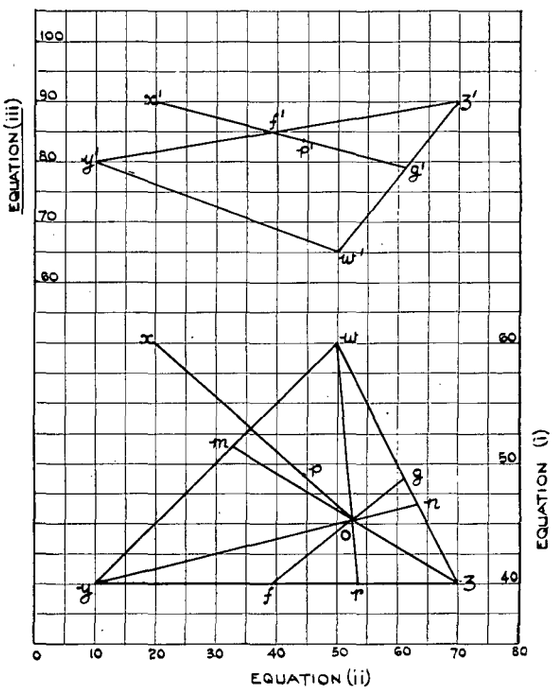

Figure 4. Example

A plane containing XP and at right angles to the vertical plane will project on the latter as $x'p'$. This line cuts the sides of triangle $y'w'z'$ at f' and g'. $f'g'$ is therefore the projection on the vertical plane of the intersection of plane YWZ and that containing XP. Because points f' and g' belong to $y'w'$ and $y'z'$, their projections on the horizontal plane, f and g, should lie on yw and yz, respectively. Point O is the intersection of XP and plane YWZ; therefore xp is extended to intersect fg at point o.

In the graphical solution described for simplicity, the vertical plane containing a and b is rotated clockwise so that the two planes are superimposed (Figure 2). It is evident that the change of scale by addition or multiplication does not alter the solution.

Example.

$$60x + 40y + 40z + 60w = 49 \quad \text{(i)}$$

$$20x + 10y + 70z + 50w = 44.5 \quad \text{(ii)}$$

$$90x + 80y + 90z + 65w = 83.5 \quad \text{(iii)}$$

$$x + y + z + w = 1 \quad \text{(iv)}$$

Plot coefficients of x, y, z, and w in (i) and (iii) against the corresponding coefficients in (ii) to obtain points x', y', z', and w', respectively (Figure 4). Points p and p' are similarly obtained by plotting 49 and 83.5 against 44.5. Produce $x'p'$ to obtain points f' and g'. Transfer these points vertically down onto the corresponding sides, yz and zw, to obtain f and g. Extend xp to intersect fg at o. The fractional concentrations of the components are then given by

$$x = (op/ox) = 22/88 = 0.25$$

$$y = (no/ny)(1 - x) = \frac{22}{110} \times 0.75 = 0.15$$

$$z = (mo/mz)(1 - x) = \frac{46.7}{87.5} \times 0.75 = 0.40$$

$$w = (ro/rw)(1 - x) = \frac{21.2}{80} \times 0.75 = 0.20$$

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RECEIVED July 21, 1950.

Colorimetric Determination of Vanadium with Benzoylphenylhydroxylamine

SUDHIR CHANDRA SHOME[1]

Indian Institute of Science, Bangalore, India

VANADIUM, as vanadate, has been determined colorimetrically using various organic reagents. The uses of strychnine (*6*, *12*), diphenylamine (*7*, *12*), and aniline hydrochloride (*14*) have been reported for the colorimetric determination of the metal. Montequi and Gallego (*9*) have prepared the compounds of vanadate ions with 8-quinolinol (8-hydroxyquinoline) and have found the violet-black precipitate obtained from a slightly acid solution to be $(C_9H_6ON)_4V_2O_3$. The same workers have separated vanadium from chromium by extracting the 8-quinolinol compound with chloroform. Bach and Trelles (*1*) have determined vanadium in water by extracting the quinolate with isoamyl alcohol. Molland (*8*) employed 8-quinolinol-5-sulfonic acid instead of 8-quinolinol. Chervyakov and Ostroumov (*3*) determined minute quantities of vanadium in uranium preparations using *p*-dimethylaminoaniline. Szebellédy and Ajtai (*13*) studied the catalytic effect (which was activated with pyrocatechol) of vanadium upon the reaction between *p*-phenetidine and potassium bromate, and determined as little as 0.0006 microgram of vanadium. Findlay and Furman (*4*, *5*) extracted vanadium even in microgram amounts from dilute sulfuric acid solution by cupferron and ether prior to its estimation by colorimetric methods.

A series of allied organic compounds was investigated (*11*) in order to improve upon the defects of cupferron (ammonium salt of nitrosophenylhydroxylamine). The use of benzoylphenylhydroxylamine, which was first prepared by Bamberger (*2*), as an analytical reagent for the gravimetric determination of copper, iron, aluminum, and titanium, has recently been described by the author (*10*). Benzoylphenylhydroxylamine, like cupferron, gives a mahogany red precipitate with vanadate ions. The precipitate is soluble in organic solvents such as ethyl alcohol, benzene, and acetic acid. This new organic reagent is not suitable for the gravimetric determination of vanadium, because a portion of the complex remains in the colloidal condition and passes through the filter paper (Whatman No. 42). In the present investigation benzoylphenylhydroxylamine was employed for the colorimetric determination of vanadium.

[1] Present address, National Institute of Sciences, Delhi 8, India.

APPARATUS AND SOLUTIONS

Absorption measurements at various wave lengths were made visually with a polarizing spectrophotometer (Gaertner), using a solution thickness of 1 cm.

Colorimetric comparisons were carried out using a Duboscq colorimeter. Sensitivity tests were performed in 50-ml. Nessler tubes. pH values of the solutions were measured with a glass electrode.

Benzoylphenylhydroxylamine Solution. A 0.2% solution of benzoylphenylhydroxylamine in ethyl alcohol was used in the spectrophotometric work.

Vanadium Solution. A vanadate solution was prepared by dissolving sodium vanadate in distilled water and the vanadium content was determined by precipitating with cupferron in ice-cold solutions. A portion of the stock solution was diluted so that the final solution contained 0.05 mg. of vanadium per ml.

Diverse Ion Solutions. Standard solutions of ferric alum and titanium sulfate were prepared separately by the usual methods. The other solutions were made by dissolving weighed amounts of salts in distilled water, each milliliter containing 2.5 mg. of the ion in question. Solutions of the anions were prepared from the alkali metal salts; sulfates were used for the solutions of the cations.

All the chemicals used were of analytical reagent quality.

SPECTROPHOTOMETRIC STUDY OF COLORED SOLUTIONS

In preparing the colorimetric solutions used in this study the following procedure was adopted.

A known amount of vanadium solution (1 to 15 ml.) was introduced into a 50-ml. volumetric flask and suitable quantities of dilute sulfuric acid were added to adjust the pH of the final solution to the required value. A preliminary experiment was carried out in a small beaker to regulate the pH of the solutions. Benzoylphenylhydroxylamine solution (10 ml.) was then added and the contents were thoroughly mixed. The resulting solution was diluted with ethyl alcohol (15 ml.) and made to volume with distilled water. After 10 minutes, the absorption due to the solution was measured with the spectrophotometer.

Effect of pH. The orange-red color formed by benzoylphenylhydroxylamine with vanadate ions was influenced by the pH of the solutions. The variation of absorbancy ($\log I_0/I$) of the colored solution, containing 10 mg. per liter of vanadium, with pH is shown in Figure 1. As the field of view was not very bright at 480 mμ, it was convenient to take the readings at 510 mμ. The

intensity of the color reached a maximum when the pH of the solution was between 1.9 and 2.8. Above pH 2.8 the color did not develop fully, while below pH 1.9 it faded gradually.

Effect of Benzoylphenylhydroxylamine Concentration. For solutions with a final volume of 50 ml., 10 ml. of benzoylphenylhydroxylamine solution (0.2%) were sufficient for the full development of color when vanadium concentrations up to 15 mg. per liter were used in the pH range of 1.9 to 2.8. The intensity of the color did not increase when more organic reagent was used.

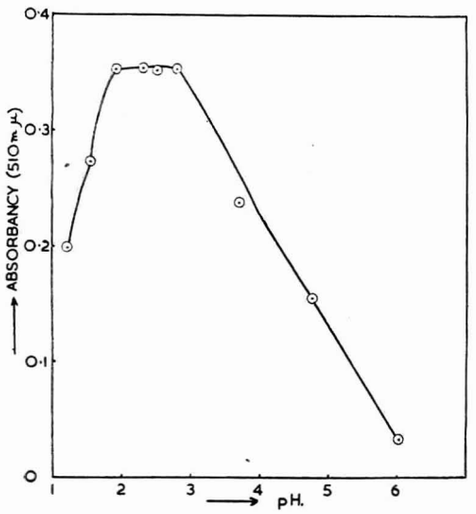

Figure 1. Effect of pH

Effect of Vanadium Concentration. Absorption curves of the colored solutions at a pH of 2.5 are shown in Figure 2. Absorption measurements were made at wave lengths of 480 to 620 mμ with solutions containing 1 to 15 mg. per liter of vanadium. Measurements at wave lengths less than 480 mμ could not be carried out with the spectrophotometer. The absorption was maximum at 480 mμ in the range of wave lengths studied, and with a solution containing 15 mg. per liter of vanadium the absorption at this wave length was 83.2%. The colored solutions obeyed Beer's law.

Stability of Color. The orange-red color formed by benzoylphenylhydroxylamine with vanadate ions was stable for about 5 hours at pH 2.6 but the color faded slowly thereafter.

Table I. Effect of Diverse Ions

Ion	Added as	Amount Permissible, Mg./L.
Fe^{+++}	$Fe_2(NH_4)_2(SO_4)_4$	0
Al^{+++}	$Al_2K_2(SO_4)_4$	0
Mn^{++}	$MnSO_4$	360
Ti^{++++}	$Ti(SO_4)_2$	50
$C_4H_4O_6^{--}$	$NaKC_4H_4O_6$	500
$C_2O_4^{--}$	$Na_2C_2O_4$	25
$HAsO_4^{--}$	Na_2HAsO_4	500
HPO_4^{--}	Na_2HPO_4	500
MoO_4^{--}	Na_2MoO_4	120
WO_4^{--}	Na_2WO_4	10
CrO_4^{--}	K_2CrO_4	15

Effect of Diverse Ions. In measuring the effect of the diverse ions, the vanadium solution containing 0.5 mg. of vanadium was taken in a 50-ml. volumetric flask and a known amount of the solution containing the ion in question was added.

To this was added dilute sulfuric or dilute ammonium hydroxide solution to maintain the pH of the final solution between 2.4 and 2.6. The benzoylphenylhydroxylamine solution (10 ml.) was added, followed by the addition of ethyl alcohol (15 ml.). The contents were thoroughly mixed and the solution was made to the mark with distilled water. After 10 minutes, the absorption due to the solution was measured at 510 mμ.

In general, the effect of 500 mg. per liter of diverse ions was studied; when interference was noticed, smaller amounts were used until the change in absorption was not more than 2% of the theory. The results are recorded in Table I.

Iron reacted with benzoylphenylhydroxylamine to give a pink colored solution and the sensitivity of this reaction was as high as that of vanadium. Titanium interfered when present in more than 50 mg. per liter, because it formed a yellow complex with the organic reagent. Aluminum, manganese, and many other ions interfered with the color reaction, probably because they reacted with the vanadate ions.

Table II. Determination of Vanadium with Benzoylphenylhydroxylamine

(Total volume of solution = 50 ml.)

Vanadium Taken, Mg.	Benzoylphenylhydroxylamine Added, G.	Vanadium Found, Mg.	Error, Mg.
0.175	0.01	0.176	+0.001
0.500	0.02	0.494	−0.006
0.650	0.02	0.660	+0.010
3.160	0.20	3.100	−0.060
6.510	0.32	6.400	−0.110

DETERMINATION OF VANADIUM USING A DUBOSCQ COLORIMETER

Vanadium was estimated with benzoylphenylhydroxylamine using a Duboscq colorimeter. The concentrations of vanadium in the sample and standard did not differ by more than 25%. The vanadium content (0.175 to 6.51 mg.) of different samples of the sodium vanadate solution was determined by the procedure described below. The results are given in Table II.

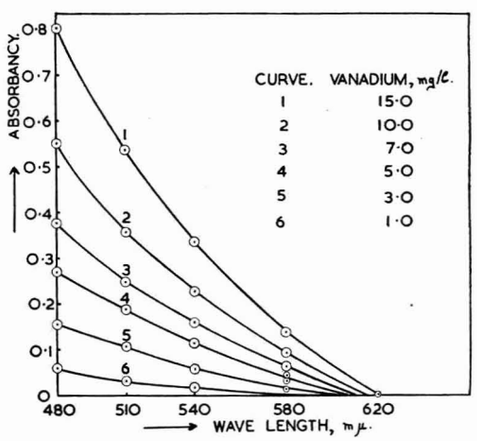

Figure 2. Absorption Curves of Vanadium-Benzoylphenylhydroxylamine Complex

In a 50-ml. volumetric flask, the vanadate solution was acidified with 0.2 to 2.0 ml. of 1 *N* sulfuric acid to adjust the pH of the final solution to approximately 2.4. A weighed excess of benzoylphenylhydroxylamine dissolved in ethyl alcohol was added, followed by further alcohol so that the final solution contained 50 to 70% alcohol. The solution was diluted to the mark with distilled water, and the color developed was compared with that of the standard prepared in a similar manner. Any unnecessary delay in the colorimetric measurements was avoided; otherwise the concentration of vanadium in the solution slowly increased owing to the evaporation of alcohol. The solution should not contain more than 6.5 mg. of vanadium, because slight precipitation of the vanadium complex occurred at higher concentrations.

Sensitivity of Color Reaction. Sensitivity measurements were carried out in 50-ml. Nessler tubes, using the same quantity of reagent solution in the blank. It was found that the smallest amount of vanadium that could be detected with benzoylphenylhydroxylamine was 0.33 mg. in 1 liter of solution.

CONCLUSIONS

Benzoylphenylhydroxylamine provides a simple and sensitive method for the colorimetric determination of vanadium. Moreover, this organic reagent can be prepared easily and preserved indefinitely. Vanadium can be estimated in presence of certain ions, but iron and aluminum, even in traces, interfere with the procedure. Further work will be required to eliminate the interferences caused by iron and aluminum which are commonly associated with vanadium.

ACKNOWLEDGMENT

The author wishes to express his sincere thanks to Sir J. C. Ghosh, director, Indian Institute of Science, for the opportunity to carry out this investigation and to S. C. Bhattacharyya for his suggestions and help.

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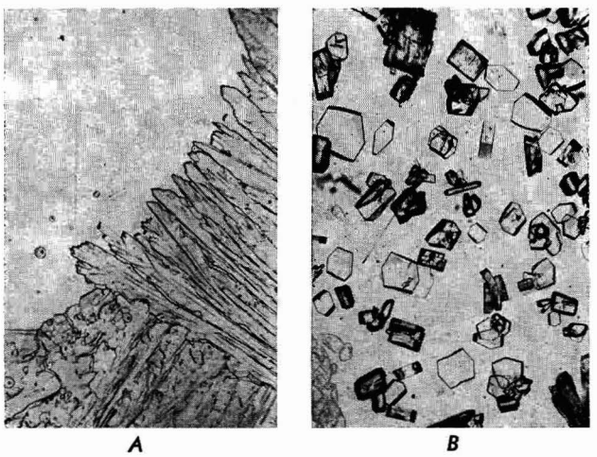
RECEIVED September 26, 1949.

CRYSTALLOGRAPHIC DATA

47. 1,8-Dinitronaphthalene I

Contributed by WALTER C. McCRONE, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

EXCELLENT crystals of 1,8-dinitronaphthalene can be obtained from alcohol or benzene. Crystals from benzene lie preferentially on the basal pinacoid. On a microscope slide thymol can be used to give perfectly formed crystals for morphological and optical study (Figure 1).



A B

Figure 1. 1,8-Dinitronaphthalene

A. **Form II growing from melt**
B. **Form I crystallized from thymol on microscope slide**

Although 1,8-dinitronaphthalene has at least two unstable polymorphic forms, the latter have been obtained only from the melt on a microscope slide.

CRYSTAL MORPHOLOGY

Crystal System. Orthorhombic.

Form and Habit. From alcohol as flat rhombs lying on the macropinacoid, {100}; the prism form, {110}, and the brachydome, {011}, are also usually present; from benzene the basal pinacoid, {001}, is the dominant form.

Axial Ratio. $a:b:c = 0.758:1:0.359$.

Interfacial Angles (Polar). $110 \wedge 1\bar{1}0 = 74°20'$; $011 \wedge 0\bar{1}1 = 39°\ 26°$.

X-RAY DIFFRACTION DATA

Cell Dimensions. $a = 11.37$ A.; $b = 15.00$ A.; $c = 5.38$ A.

Formula Weights per Cell. 4; 3.99 (calculated from x-ray data).

Formula Weight. 218.16.

Density. 1.587 (flotation and pycnometer); 1.591 (calculated from x-ray data).

Principal Lines

d	I/I_1	d	I/I_1
7.52	0.3	2.85	0.6
6.27	0.8	2.77	0.8
5.72	0.8	2.71	Very weak
5.34	0.1	2.67	Very weak
5.09	0.2	2.64	Very weak
4.87	0.2	2.55	Very weak
4.63	0.7	2.48	0.3
4.34	Very weak	2.44	0.2
4.11	0.2	2.38	Very weak
3.92	0.2	2.33	Very weak
3.80	0.9	2.26	0.5
3.67	Very weak	2.22	Very weak
3.47	0.2	2.19	Very weak
3.08	0.1	2.14	Very weak
3.04	1.0	1.89	0.05
2.97	Very weak	1.87	0.05

OPTICAL PROPERTIES

Refractive Indexes (5893 A.; 25° C.). $\alpha = 1.634 \pm 0.002$. $\beta = 1.763 \pm 0.005$. $\gamma = 1.86$ (calculated from α, β, and $2V$).

Optic Axial Angles. (5893 A.; 25° C.). $2V = 80°$.

Dispersion. Very strong, $v > r$.

Optic Axial Plane. 001.

Sign of Double Refraction. Negative.

Acute Bisectrix. $\alpha = a$.

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.750$. R(calcd.) = 54.1. R(obsd.) = 56.0.

FUSION DATA. 1,8-Dinitronaphthalene melts at 170–172° C. with neither sublimation nor decomposition. When completely melted it solidifies quickly on cooling to give either Form II or

III. Form III is formed if the preparation is chilled rapidly, although some Form II is almost always present. Form III forms long broad rods with unique mechanical twin bands forming on cooling (these disappear on heating and reappear on cooling). The interference figure is either an optic normal or an obtuse bisectrix figure. Occasionally a rhomb-shaped crystal appears embedded in these rods. The rhomb shows no inclination to transform or grow and may be another view of Form III (if not, it is Form IV). It shows a centered BX_a figure, $2E$ ca. 60°, $v > r$, (−); the optic axial plane bisects the acute profile angle of 55°.

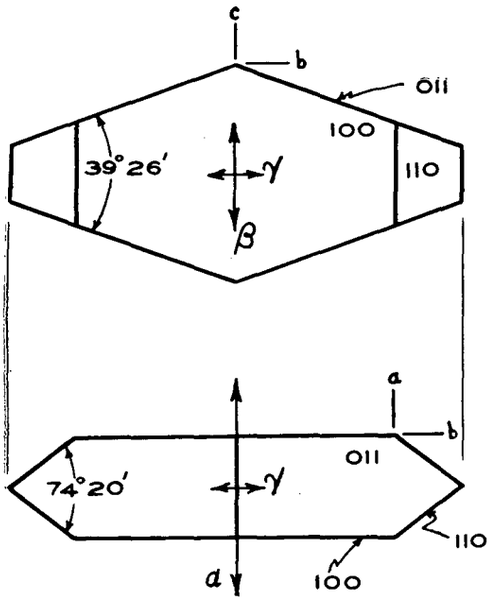


Figure 2. Orthographic Projection of Typical Crystal of 1,8-Dinitronaphthalene
As recrystallized from thymol on a microscope slide

The crystals from the melt nearly always show some Form II and never show Form I. Form II is characterized by large globular areas of uniform polarization color, often a deep blue or purple. The latter corresponds to the centered acute bisectrix view. The figure shows crossed axial plane dispersion with $2E$ very low, (−).

Form I cannot be obtained from the melt. All attempts lead to Form II, since Form I changes to Form II at the transition temperature of about 110° C. Recrystallization from thymol leads often to a rather startling solid solid transformation, probably of Form III to II. Long rods grow as the melt cools until suddenly each gives a tremendous shiver as it twists and bends during transformation.

1,8-DINITRONAPHTHALENE II

CRYSTAL MORPHOLOGY.

Crystal System. Orthorhombic.

Form and Habit. Crystallizes from thymol on a microscope slide as rhombs similar in appearance to Form I, except that the rhomb profile angle is 55° and that view shows crossed axial plane dispersion.

OPTICAL PROPERTIES.

Dispersion of Optic Axes of 1,8-Dinitronaphthalene II

Wave Length, A.	$2E$	Wave Length, A.	$2E$
6350	27°	5370	0°
6140	25°	5290	−11°
5930	23°	5070	−18°
5720	20°	4860	−32°
5500	11°		

Sign of Double Refraction. Negative.

1,8-DINITRONAPHTHALENE

At least one additional unstable modification is obtained on crystallization from the melt or from thymol solutions on a microscope slide. Everything known about this very unstable form is included under fusion data for Form I.

ACKNOWLEDGMENT

This description is based on preliminary work carried out at Cornell University under contract OEMsr-193 with the OSRD during 1943–44 under the administrative direction of Alfred T. Blomquist. John H. Andreen and Sien-Moo Tsang were associated with this project and contributed to the above description.

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, supervisor, Analytical Section, Armour Research Foundation of the Illinois Institute of Technology, Chicago, Ill

BOOK REVIEWS

Metal Spectroscopy. *F. Twyman.* vii + 569 pages. Charles Griffin & Co., Ltd., 42 Drury Lane, London WC2, England, 1951. Jarrell-Ash Co., Boston, Mass. Price, $8.75.

Although ostensibly a comprehensive treatise on the spectrochemical analysis of metals, this work is especially interesting and enjoyable as a character sketch of the author and as an outline of his more than fifty years of outstanding contribution to this field. Essentially British, slightly provincial, and markedly Hilgeresque, the book provides an excellent historical background and is replete with lore of spectroscopy of earlier times. The development of the art and science of emission spectroscopy up to about 1945 is covered to an excellent degree of completeness and expertness. The treatment of developments from 1945 up to the date of publication is rather fragmentary, incomplete, and rather uncritical with the exception of the several narrow fields covered by the collaboratory authors. The book can be regarded as representing authoritative British opinion as of 1945 plus a certain amount of selected descriptive material on later developments.

Twyman has made an obvious and generally successful attempt to be fair and objective in his comparative treatment of commercial instruments. His frank and frequent use of the first person singular and the frequency of such phrases as "when I designed the instruments from which all modern quartz spectrographs for metallurgical work are derived" are probably more than justified by historical fact. As a loyal member and leader of the old guard, Twyman is still fighting the battle of the prisms versus the gratings and is not yet ready to grant the commercial supremacy of gratings so evident in the United States. His former opposition to microphotometers as practical analytical devices has almost completely disappeared and there remains in this book only a nostalgic comment on the failure of the logarithm sector to obviate the necessity for such instruments.

American readers may be annoyed by the author's continued adherence to galvanometer deflection ratios in place of the more elegant and precise methods of calibration generally used in the U.S.A. The latter are described in brief, but the author implies that he believes most practical analysts restrict measurements to the "straight-line" portion of the emulsion calibration curve.

The analyst interested in nonroutine applications will find much of interest in the chapter "Spectrochemical Analysis in Works Practice." Many practical methods are described in detail and some material is included on virtually all types of materials analyzed spectroscopically. Unfortunately, many of the most advanced methods, notably those developed in the U.S.A. for aluminum alloys, steel, and zinc alloys, are omitted or treated very superficially.

The principal failings of this book are in the treatment of techniques and instruments which have come into prominence during the past six or seven years. These deficiencies are more than compensated for by the wealth of interesting and instructive material presented. This book should be read by everyone working in the field of spectrochemical analysis.

J. R. CHURCHILL

Métodos de Análisis Químico Unifixados (Recommended Methods of Chemical Analysis). Part I. 163 pp. Inst. Hierro y Acero, Madrid, Spain, 1950.

A committee was elected to compile and unify the various methods used to determine elements present in iron alloys. General specifications for reagents and sampling are briefly presented for steel, pigs, and cast irons. In several instances various techniques are given for the determination of carbon, sulfur, phosphorus, manganese, silicon, chromium, nickel, molybdenum, tungsten, vanadium, copper, cobalt, aluminum, and tin. In a series of five appendixes the following topics are covered: a discussion of the fundamental principles behind the above determinations; a brief review of errors and precision of chemical analytical methods; rapid analysis, such as the drop methods and electrographic methods; and physicochemical methods of analysis, such as spectrochemical, photometric, and potentiometric methods. The latter are very little used in Spain at the present. The bibliography contains over 50 items. On the last page are listed the known errata. F. R. MORRAL

Documents d'Analyse Chimique. Analyse des Silicates, Roches, Verres, Couvertes, Refractaires, etc. *Arnold Lassieur.* viii + 184 pages. Dunod, 92, Rue Bonaparte, Paris VI^e, France, 1951. Price, 680 francs.

This little book should be entitled "Notes on the Analysis of Silicates." The author discusses preparation of the sample, then devotes chapters to: a review of classical methods for silica, the R_2O_3 separation iron, alumina, titania, lime, magnesia, and the alkalies. He then gives in considerable detail his preferred procedure for analysis of such silicates. His procedure differs from standard American practice in several ways—for example, a single dehydration for silica, a single R_2O_3 precipitation, and the use of cupferron to precipitate aluminum.

Brief discussions are given in other chapters of methods for elements such as barium, lead, lithium, etc., that occur in some silicates. Throughout the book the author gives considerable experimental data to support his conclusions. Though references to the literature since 1935 are rare, with the exception of the work of Duval on the thermobalance, chemists analyzing silicates will find this book interesting even if they do not always agree with the author's conclusions. ROBERT FOWLER

Standard Methods for Testing Petroleum and Its Products. 11th ed. 724 pages. Institute of Petroleum, 26 Portland Place, London, W I, England, 1951. Price, 31s. 0d.

The eleventh edition of "Standard Methods" is an excellent complement to the "A.S.T.M. Standards on Petroleum Products," both of which are essential to the petroleum analyst.

As the technology of petroleum advances from year to year, the number of petroleum products steadily increases, together with more rigid specifications for each, so it becomes necessary to revise, improve, standardize, and consolidate the various analytical techniques. This is reflected in the increased size of the new edition. The technology of petroleum has advanced at such a rapid pace it cannot be expected that either the "A.S.T.M. Manual" or "Standard Methods" contains everything. Therefore, the petroleum analyst will find it advantageous to use one in conjunction with the other.

"Standard Methods" has one distinct advantage over "A.S.T.M." in that its indexes are more concise and understandable, making the problem of method selection somewhat easier.

The section titled "New and Revised Methods, Specifications, etc." tells at a glance most of the changes made in the new edition over the older one.

A comparison of various test methods contained in both "A.S.T.M." and "Standard Methods" indicates that standardization has been the keynote of the new edition, as evidenced by the large number of cross-referenced numerical listings. For example, I.P.-128–51 (T), a new test for aromatic content, is based on the procedure for aromatic content in A.S.T.M. D 875–46T.

It is apparent that close cooperation between A.S.T.M. Committee D-2, Natural Gasoline Association of America, and I.P.'s Standardization Committee has resulted in the merger of the best merits of various tests. R. E. MEYER

Quantitative Analysis, A Theoretical Approach. *William Rieman, III, Jacob D. Neuss,* and *Barnet Naiman.* 3rd edition. x + 523 pages. McGraw-Hill Book Co., New York 18, N. Y., 1951. Price, $5.

This is a revised and enlarged version of the second edition, which appeared in 1942. A more detailed discussion of the general quantitative laboratory techniques is given, some new illustrations have been added, and the directions for many of the laboratory exercises have been improved. The chapter on photometric methods now includes the theory and use of photoelectric instruments, of both the one-cell and the two-cell types. A chapter has been added on the theory and use of ion exchange as an analytical technique, a novel feature that should be of considerable interest. A practical exercise on the determination of arsenic in insecticides is included in the chapter. This textbook should appeal strongly to teachers of elementary quantitative analysis who wish to give a thorough course with emphasis on modern theories. The binding is attractive, the format is pleasing, and the text appears to be remarkably free from typographical errors. EARLE R. CALEY

Analytical Division Speakers

THE Speakers Procurement Committee of the Division of Analytical Chemistry has prepared the following list of speakers on analytical subjects for 1951–52.

1. Absorption Spectroscopy

T. MOELLER, University of Illinois, Urbana, Ill.	Absorptiometric Determination of Rare Earth Elements Some Analytical Aspects of 8-Quinolinol Chelates Spectrophotometric Characteristics of 8-Quinolinol Chelates
M. G. MELLON, Purdue University, Lafayette, Ind.	Analytical Spectrophotometry The Role of Heteropoly compounds in Absorptiometry Chemistry, Curves, and Color
R. R. BRATTAIN, Shell Development Co., Emeryville, Calif.	Absorption Spectroscopy
G. H. AYRES, University of Texas, Austin, Tex.	Fundamentals of Spectrophotometry

2. Application of Analytical Chemistry to Archeology

E. R. Caley, Ohio State University, Columbus, Ohio	Archeological Chemistry Application of Chemistry to Archeology Chemical Analysis of Ancient Material Modern Chemical Analysis and Ancient Chemical Technology Density as an Index of the Composition of Ancient and Modern Alloys
M. Farnsworth, Metal & Thermit Corp., New York, N. Y.	Archeological Chemistry

3. Chromatography

H. H. Strain, Argonne National Laboratory, Chicago, Ill.	Chromatography Separation Based on Electromigration in Chromatographic Systems
A. Rose, Pennsylvania State College, State College, Pa.	Predicting Composition Changes in Selective Absorption of Liquid Mixtures

4. Determination of Organic Functional Groups

S. Siggia, General Aniline and Film Co., Easton, Pa.	Quantitative Organic Analysis via Functional Groups
J. Mitchell, Jr., E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.	Organic Functional Group Analysis

5. Distillation

E. S. Perry, Distillation Products, Inc., Rochester, N. Y.	Some Analytical Applications of Molecular Distillation
A. Rose, Pennsylvania State College, State College, Pa.	Analytical Distillation Procedures and Apparatus Separations by Vacuum Distillation
W. J. Podbielniak, Podbielniak, Inc., 341 East Ohio St., Chicago 11, Ill.	Recent Advances in Analytical and Laboratory Distillation Theory and Technique of Testing High-Efficiency Laboratory Columns

6. Electrometric and Related Methods

W. H. MacNevin, Ohio State University, Columbus, Ohio	Developments in Coulometric Analysis
N. H. Furman, Princeton University, Princeton, N. J.	Coulometric Methods
L. B. Rogers, Massachusetts Institute of Technology, Cambridge, Mass.	Electrolyses at Controlled Potentials
P. J. Elving, Pennsylvania State College, State College, Pa.	Electrochemical Carbon-Halogen Bond Fission
D. D. DeFord, Northwestern University, Evanston, Ill.	Coulometric Titrations
H. A. Laitinen, University of Illinois, Urbana, Ill.	Amperometric Titrations
E. H. Swift, California Institute of Technology, Pasadena 4, Calif.	Coulometric Methods of Analysis
W. D. Cooke, Cornell University, Ithaca, N. Y.	Coulometric Methods of Analysis

7. Electron Microscopy

L. Marton, National Bureau of Standards, Washington, D. C.	Electrons *vs.* Photons. A Comparison of Microscopes Measuring with the Electron

8. Emission Spectroscopy

L. W. Strock, Saratoga Springs Commission, Saratoga Springs, N. Y.	Direct Current Arc Methods Spectrochemical Determination of Major Constituents in Silicate and Refractory Materials Trace Element Analysis of Biological Materials

9. Extraction

N. H. Furman, Princeton University, Princeton, N. J.	Analytical Extraction
Lyman C. Craig, Rockefeller Institute for Medical Research, New York 21, N. Y.	Characterization of Substances by Extraction
W. J. Podbielniak, Podbielniak, Inc., Chicago, Ill.	Centrifugal Countercurrent Liquid-Liquid Contacting
G. H. Morrison, U. S. Atomic Energy Commission, New Brunswick, N. J.	Solvent Extraction, a New Analytical Tool

10. Fluorescence Methods

W. F. Neuman, University of Rochester, Rochester, N. Y.	Fluorescence Analysis, an Art or a Science
C. E. White, University of Maryland, College Park, Md.	Inorganic Fluorometric Analysis

11. Fundamentals of Analytical Chemistry

W. M. MacNevin, Ohio State University, Columbus, Ohio	Educational Trends in Analytical Chemistry
H. H. Willard, University of Michigan, Ann Arbor, Mich.	Importance of Analytical Chemistry in Our Industrial Age (Nontechnical)
W. C. McCrone, Armor Research Foundation, Chicago 16, Ill.	Teaching *vs.* Practice of Analytical Chemistry
L. Lykken, Shell Development Co., Emeryville, Calif.	Teaching of Analytical Chemistry in Colleges Analytical Chemistry as a Profession

12. Gas Analysis

Leonard K. Nash, Harvard University, Cambridge, Mass.	Recent Advances in Gas Analysis

13. Gravimetric and Volumetric Analysis

W. M. MacNevin, Ohio State University, Columbus, Ohio	Reactions and Uses of Lithium Aluminum Hydride
H. H. Willard, University of Michigan, Ann Arbor, Mich.	Separation by Precipitation from Homogeneous Solutions Determination of Fluoride Use of Ceric Salts in Analytical Chemistry
P. J. Elving, Pennsylvania State College, State College, Pa.	Role of Heterogeneous Equilibria in Analytical Chemistry
J. Mitchell, Jr., E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.	Analytical Applications of the Karl Fischer Reagent

14. Infrared Spectroscopy

R. R. Brattain, Shell Development Co., Emeryville, Calif.	Absorption Spectroscopy
E. J. Rosenbaum, Sun Oil Co., Norwood, Pa.	Analytical Applications of Molecular Spectroscopy

15. Instrumental Analysis

J. Mitchell, Jr., E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.	Instrumental Analysis
L. Lykken, Shell Development Co., Emeryville, Calif.	Approach to Analysis in a Modern Industrial Laboratory
D. J. Pompeo, Shell Development Co., Emeryville, Calif.	Instrumentation

16. Ion Exchange Methods of Analysis

W. M. MacNevin, Ohio State University, Columbus, Ohio	Separation of Platinum Group Metals with Ion Exchange Resins
R. Kunin, Rohm and Haas Co., Philadelphia, Pa.	Ion Exchange in Analytical Chemistry
W. Rieman, III, Rutgers University, New Brunswick, N. J.	Ion Exchange in Analytical Chemistry

G. E. Boyd, Oak Ridge National Laboratory, Oak Ridge, Tenn.	Ion Exchange Separations
E. R. Tompkins, U. S. Naval Radiological Defense Laboratory, San Francisco 24, Calif.	Ion Exchange Methods of Analysis

17. Mass Spectroscopy

Alfred O. C. Nier, University of Minnesota, Minneapolis, Minn.	The Mass Spectrometer as a Scientific and Industrial Research Tool
F. J. Norton, General Electric Co., Schenectady, N. Y.	Applications of the Mass Spectrometer
H. W. Washburn, Consolidated Engineering Corp.	Mass Spectroscopy
J. A. Hipple, National Bureau of Standards, Washington 25, D. C.	Recent Developments in Analytical Mass Spectroscopy
	Analysis of Solids with the Mass Spectrometer

18. Microchemistry

A. A. Benedetti-Pichler, Queens College, Flushing, N. Y.	Microscopy of Organic Substances on Hot Stage with the Technique of the Koflers
	Microgram Techniques of Chemistry
	Microtitrimetry
	Principles for the Development of Micromethods
J. Mitchell, Jr., E. I. du Pont de Nemours & Co., Inc., Wilmington, Del	Microscopic Identification of Organic Compounds

19. Nucleonics

G. E. Boyd, Oak Ridge National Laboratory, Oak Ridge, Tenn.	Radioactivity and Analytical Chemistry
S. A. Reynolds, Oak Ridge National Laboratory, Oak Ridge, Tenn.	Neutron Radioactivation Analysis

20. Organic Analytical Reagents

Frank Welcher, University of Indiana, Bloomington, Ind.	Organic Analytical Reagents

21. Polarography

W. M. MacNevin, Ohio State University, Columbus, Ohio	Organic Polarography
L. B. Rogers, Massachusetts Institute of Technology, Cambridge, Mass.	Polarography Using Solid Electrodes
I. M. Kolthoff, University of Minnesota, Minneapolis, Minn.	Fundamentals of Polarography and Amperometric Titrations
P. J. Elving, Pennsylvania State College, State College, Pa.	Polarographic Behavior of Organic Compounds
H. A. Laitinen, University of Illinois, Urbana, Ill.	Polarography in Liquid Ammonia
	Recent Polarographic Studies of Inorganic Complexes

22. Raman and Ultraviolet Spectroscopy

E. J. Rosenbaum, Sun Oil Co., Norwood, Pa.	Recent Developments in Raman Spectroscopy
	Analysis by Ultraviolet Absorption Spectroscopy
B. F. Dubenbostel, Jr., Esso Laboratories, Standard Oil Development Co., Linden, N. J.	Application of Raman Spectroscopy to the Analysis of Petroleum Products

23. Reaction Kinetics in Analytical Chemistry

I. M. Kolthoff, University of Minnesota, Minneapolis, Minn.	Reaction Kinetics in Analytical Chemistry

24. Standardization of Analytical Methods

F. D. Tuemmler, Shell Development Co., Emeryville, Calif.	Standardization of Analytical Methods
	Analytical Standardization Within a Petroleum Company
	Format Requirements of a Published Standard Method

25. Statistical Quality Control

G. Wernimont, Eastman Kodak Co., Rochester, N. Y.	Statistical Methods in Chemistry

26. Ultrasonics and High Frequency Titrations

F. W. Jensen, Texas A. & M. College, College Station, Tex.	Analysis in High Frequency Fields
W. J. Blaedel, University of Wisconsin, Madison, Wis.	High Frequency Titrations

27. X-Ray Methods

H. A. Liebhafsky, General Electric Co., Schenectady, N. Y.	X-Ray Methods in Chemical Analysis
William Parrish, Phillips Laboratories, Inc., Irvington-on-Hudson, N. Y.	Chemical Analysis by X-Ray Powder Diffraction

28. Miscellaneous

L. A. Wooten, Bell Telephone Laboratories, Murray Hill, N. J.	Analytical Chemistry in Communications Research
A. Rose, Pennsylvania State College, State College, Pa.	Automatic Computers in Chemical and Engineering Research
M. G. Mellon, Purdue University, Lafayette, Ind.	Analytical Automatons
	Chemical Writing
A. A. Benedetti-Pichler, Queens College, Flushing, N. Y.	Science as an Ethical Force
	Austria, Its Past, Present, and Future
	Theory of Sampling (general mathematical treatment)
W. C. McCrone, Armour Research Foundation, Chicago 16, Ill.	Crystallography
S. B. Knight, University of North Carolina, Chapel Hill, N. C.	Mineral Analysis and Interference Studies with the Flame Photometer
M. Farnsworth, Metal and Thermit Corp., New York 17, N. Y.	Analytical Chemistry in the Tin Industry

The Analyst's Calendar

Correction. In the calendar of coming meetings, an International Congress on Analytical Chemistry to be held in Great Britain in August was erroneously listed. The international congress sponsored by the international union is to meet in Oxford, England, beginning September 2, 1952.

World Chemical Conclave

American Chemical Society. 75th Diamond Jubilee Meeting, New York, N. Y., September 3 to 7

XIIth International Congress of Pure and Applied Chemistry. New York, N. Y., September 10 to 13

Scientific Apparatus Makers Association. Laboratory Equipment Section, Three Lakes, Wis., August 29 to September 1.

Recorder-Controller Section, Absecon, N. J., October 9 to 12.

Industrial Instrument Section, Absecon, N. J., October 18 and 19.

Laboratory Apparatus, Optical, Nautical, Aeronautical and Military Instrument Sections, New York, N. Y., November 28 to 30

AIDS FOR THE ANALYST . . . .

Confined Spot Filtration Apparatus. Jack L. Lambert[1], Thomas E. Moore, and Paul Arthur, Oklahoma A. & M. College Stillwater, Okla.

THE apparatus described by Franke and coworkers (*1*) for the colorimetric comparison of spots formed by the deposition of small amounts of colored precipitates onto a suitable background material has been modified for greater convenience of operation. In attempting to use Franke's apparatus for the determination of trace quantities of selenium (*2*), two difficulties were encountered: the filter paper had to be cut to the exact size needed, and much care had to be taken to avoid damaging the colored spot while removing the filter paper from the apparatus. The apparatus illustrated allows the use of filter paper of varying sizes and shapes, and is easily disassembled to remove the filter paper and spot after it has been formed.

The support for the filter mat is a porous borosilicate glass disk, *D*, formed in one of two 18/9 borosilicate glass socket joints, *E*, by sintering powdered borosilicate glass (passed by a 100-mesh, but retained by a 140-mesh screen) in a 2- or 3-mm. layer. A plug of asbestos wool, pressed into the joint wet and then dried in an oven, holds the powdered glass while it is being sintered. The asbestos can be easily removed after the porous disk has been formed by soaking with water and loosening with a wire probe. The sintering is done with the socket joint in an upright position, using a Meker-type burner which provides a flame hot enough to sinter the powdered glass but not hot enough to deform the joint. It is best to build up the disk in thin layers, making certain that it is firmly welded to the inside surface of the socket joint.

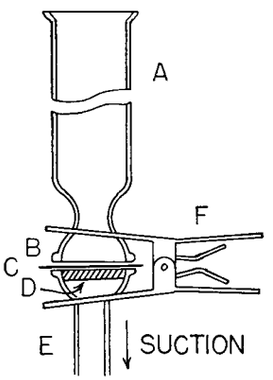


Figure 1

A 25 × 100 mm. borosilicate glass test tube, *A*, was welded as close as possible to the other socket joint *B*. The lips of both joints were ground flat and smooth on a Carborundum stone to make a tight seal to the filter paper when clamp *F* is put in place. Each joint then had an inside diameter of 18.0 mm. Decreasing the diameter of the filtration area would increase the sensitivity, but use of smaller socket joints would cause the constriction to be smaller where joint *B* is attached to test tube *A*. By using the 18/9 size socket joints, the effect of the constriction upon the filtration of fine precipitates is made negligible.

Although several qualities of filter paper were tried, the most successful was Whatman's No. 50. With a dense, hard, smooth paper such as this, the air leak around the edge was negligible even when strong suction was applied. Barium sulfate is the most generally useful background material upon which to deposit colored precipitates because of its inertness, white color, and small particle size when freshly precipitated from dilute solution. A mat of precipitated barium sulfate was prepared by mixing 10 ml. of 0.5% barium chloride solution with 10 ml. of 0.25% sulfuric acid and filtering with suction through a moistened piece of Whatman's No. 50 filter paper, *C*, in the apparatus described (Figure 1). This mat will retain on its surface fine precipitates formed in the procedures for confined spot methods of analysis. Other materials can be used to form the mat and where adsorption or chemical reaction can be employed, substances of a colloidal or ionic nature may be removed from solution.

[1] Present address, Department of Chemistry, Kansas State College, Manhattan, Kans.

With the arrangement described, all but those coarse precipitates that rapidly settle out under the influence of gravity give uniformly colored spots. Fine suspensions are deposited evenly over the surface from center to edge by the uniform pressure differential through the barium sulfate mat, filter paper, and sintered disk. If the colored spot thus formed was washed with a saturated solution of magnesium sulfate, the fragile barium sulfate mat, after drying, was cemented both to the colored surface layer and to the filter paper.

ACKNOWLEDGMENT

The research, of which the development of this apparatus formed a part, was made possible by a grant from the National Institutes of Health, United States Public Health Service, through the Research Foundation of the Oklahoma A. & M. College.

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(1) Franke, Burris, and Hutton, IND. ENG. CHEM., ANAL. ED., **8**, 435 (1936).
(2) Lambert, Moore, and Arthur, ANAL. CHEM., **23**, 1101 (1951).

Automatic Test Tube Washer. R. E. Parks, Jr., G. W. Kidder, and Virginia C. Dewey, Biological Laboratories, Amherst College, Amherst, Mass.

A MAJOR problem faced by laboratories employing chemically clean glassware is the time involved in the cleaning of test tubes with acid-dichromate cleaning solution and the subsequent rinsing.

Jordan [IND. ENG. CHEM., ANAL. ED., **17**, 270 (1945)] has described an automatic pipet washer, now in common use in many laboratories, which rinses pipets thoroughly by means of repeated fillings and drainings of the tank in which the pipets are placed. This is performed automatically through a siphon system. Unfortunately, this type of device cannot be used for the rinsing of test tubes, because the tubes are not open on both ends. If the tubes are placed in a horizontal position in a pipet washer, only slight variations from the exact horizontal would allow either air or acid to be trapped in the tubes. The authors have recently developed an apparatus (the Amherst automatic test tube washer) which accomplishes thorough cleaning and rinsing of a large number of test tubes with a minimum of handling. The new feature of this device consists of a swinging tray suspended in a rinsing tank in which a basket containing the test tubes is placed. By means of a float the tray and basket are rocked backward when the tank is filling and forward when it is draining, thus allowing the tubes to fill and drain completely. The filling and draining are controlled by a siphon system as in the Jordan pipet washer; this could be accomplished, perhaps more efficiently, by means of a solenoid valve.

The apparatus, all parts of which are made of stainless steel, is shown in Figure 1 with the side of the tank removed to show the swinging tray and basket as if the tank were full. The rinsing tank, 10.5 × 13 inches, has a water inlet and siphon system. The siphon is constructed of an outer pipe 16 inches long and 3 inches in diameter, *A*, the top of which is sealed by a 0.5-inch dome and the bottom of which has a 0.75-inch opening into the bottom of the tank. Inside the lower end of *A* is a threaded collar. Through this collar and into the outer pipe fits a 23-inch section of 1.5-inch outer diameter pipe, *B*. An elbow sleeve with a tapered end covers the lower end of the inner pipe to improve the operation of the siphon; the swinging tray, *C*, 5.5 × 7 × 12 inches, has an attached float, *D*, and adjustable bearings, *E*. The

bearings fit into bearing cylinders of thin tray supports, *F*, which suspend the tray in the rinsing tank; the basket, *G*, 11.75 × 5.375 × 12.25 inches, is made of perforated stainless steel with flanges to take a sliding screen, *H*, the top of which is provided with a handle with three notches. This basket holds about 400 15 × 125 mm. tubes; the stainless steel tank, 8 × 13 × 18 inches, contains the acid-dichromate cleaning solution.

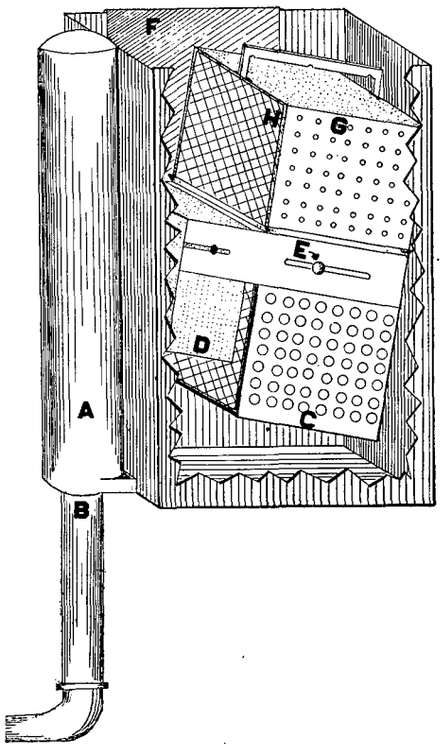

Figure 1.

The tubes are placed in the basket with their mouths toward the open side. The screen is slipped into place and the basket is immersed in the tank of cleaning fluid, inclined so that the tubes will fill without trapping air. After the required soaking time, the basket is lifted from the cleaning solution in an inclined position, and the fluid is allowed to drain from the tubes. This operation is most easily accomplished by means of a hook attached to a chain rigged from a pulley above the tank. The three notches in the handle of the basket are designed to allow the different inclinations while the basket is being immersed or removed from the cleaning tank. The basket is now lowered into the swinging tray. The tray bearings have been adjusted previously, so that the mouths of the tubes are downward when the tank is empty.

The water is started flowing into the tank. The water level raises the float, tipping the tray and basket backward, thus allowing the tubes to fill without trapping air. As the water level reaches the siphon height, all the tubes are full. At this level the siphon starts and the tank is drained rapidly. As the water level falls, the tray tips forward so that all the tubes drain. The siphon "breaks" and the tank starts filling again. This cycle will repeat itself indefinitely. After ten or more cycles have been completed, a few milliliters of saturated sodium hydroxide are added and the tank is filled to a point just below that at which the siphon starts. After sufficient soaking, the water inflow is resumed for the desired time. Distilled water may be poured into the tank for the final rinses. The basket is removed from the tank, drained, and placed in a drying oven with the mouths of the tubes downward.

In practice it is efficient to have two or more baskets; a second basket may be filled with tubes while the first is being washed. The dimensions noted are those necessary for the cleaning of 15 × 125 mm. test tubes. If tubes of other sizes are employed, the dimensions of the apparatus should be adjusted accordingly.

Cell for Rapid Polarographic Analysis. Louis Meites and Thelma Meites, Sterling Chemistry Laboratory, Yale University, New Haven, Conn.

THE difficulty of deaerating a solution rapidly, and the possibility of introducing contaminants from the agar bridge, have led many polarographers to forego the use of H-cells (*3*) in favor of less versatile cells with mercury pool anodes. Based on the cell design of Laitinen and Burdett (*2*) in which the gas stream is dispersed by a fritted-glass disk, and the older design by Carritt (*1*) which eliminated the effects of contact with the agar plug, a modified H-cell which is free from these disadvantages has been designed.

Referring to Figure 1, the gas entering at *a* enters the solution through a sintered borosilicate glass gas dispersion cylinder, *b*, which brings the gas stream into intimate contact with the solution. When deaeration is complete, the gas is diverted over the solution in compartment *c*, and tube *d* is filled by suction at *a*. Any reaction then occurring at the agar-solution interface is isolated from the main body of the solution until the reaction products diffuse through the entire length of *d*, and contamination of the solution is thereby effectively prevented. The *iR* correction corresponding to the resistance of the solution in *d* may generally be ignored, or it may be determined and applied in precise work.

When contact with an agar bridge is not harmful, a more conventional design is used, in which the gas-entry tube of the usual H-cell (*3*) is replaced by a gas-dispersion cylinder, ring-sealed through the wall of the solution compartment opposite the bridge, and positioned as shown in Figure 1.

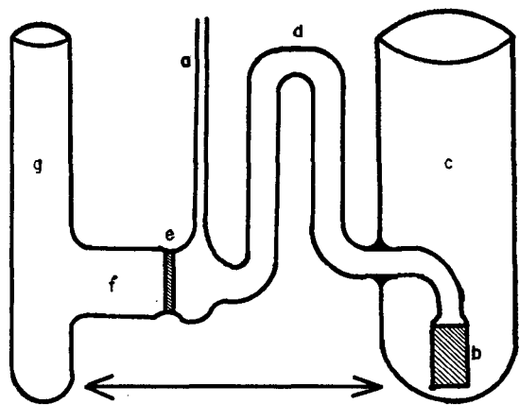

Figure 1. Modified H-Cell

a. Gas-entry tube, 4-mm. outside diameter
b. Corning 39533 12C gas dispersion cylinder
c. Solution compartment, 45 mm. outside diameter × 12 cm.
d. 9 mm. outside diameter × 18 cm. total length
e. Corning 39570 20M fritted disk
f. 25 mm. outside diameter × 3 cm.
g. Reference electrode compartment, 22 mm. outside diameter × 12 cm.
Length of arrow corresponds to 10.0 cm.

When 75 ml. of air-saturated 3 *M* potassium chloride in a cell like that shown in Figure 1 were deaerated by a rapid stream of hydrogen, the diffusion currents at −1.6 volts *vs.* the saturated calomel electrode indicated that oxygen removal was 84% complete in 20 seconds and 98% complete in 40 seconds; oxygen could not be detected after 1 minute.

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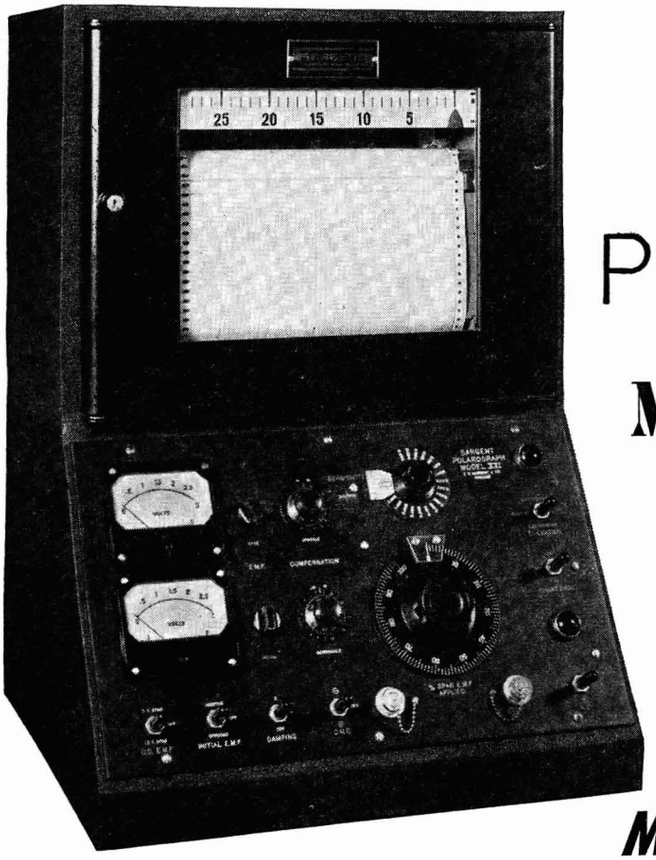
(1) Carritt, D. C., Ph.D. thesis, Harvard University, 1947.
(2) Laitinen, H. A., and Burdett, L. W., ANAL. CHEM., **22**, 833 (1950).
(3) Lingane, J. J., and Laitinen, H. A., IND. ENG. CHEM., ANAL. ED., **11**, 504 (1939).

Work supported by Contract AT(30-1)-842 between the Atomic Energy Commission and Yale University.



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INSTRUMENTATION

Use of an electric analog device is suggested for helping to solve questions of purity and characterization, constantly confronting the practicing analyst

by R. H. Müller

Two questions which constantly confront the practicing analyst are those relating to purity and characterization. The first of these, purity, has been the subject of more learned discussion, as well as futile semantic disputation, than almost any other topic. The semanticist at once brings up the question of the single foreign molecule, the insidious presence of which rules out all further discussion. The practical analyst, however, cannot afford to close up shop so early in the day and seeks criteria which are acceptable in the light of the intended use of the substance.

Criteria of Purity

The more one looks into specific cases, the more evident it becomes that the only useful criterion of purity is the designation of an upper limit of contamination, of a specific impurity, which shall be acceptable in a specific application. For example, there are many criteria for the purity of water. One can short-circuit the semanticist's field day by admitting at the outset that the possibilities of differences in isotopic composition are many. On this score, we may decide something at least from precise density determinations. These are extraordinarily sensitive, but not specific. Mass spectrographic analyses are specific but not so precise. When the contaminant is a solute, we have additional criteria, each of which is useful only in the light of the intended application. The physical chemist is apt to choose specific conductance because it can be measured with such high precision, but his acceptable sample might turn out to be a bacteriologist's delight because of all the fascinating flora to be found therein. When subject to further purifications until the bacteriologist is satisfied, the clinician might object to the sample because, when injected in an experimental animal, it gave evidence of the presence of pyrogens. With respect to dissolved gases, one encounters another set of criteria. One part of oxygen dissolved in several million parts of water may be deleterious in a steam boiler or profoundly modify the course of a biochemical reaction.

Whether one uses a chemical method of analysis such as Winkler's method, or instrumental criteria such as polarographic reduction or magnetic susceptibility, the purity test is largely evaluated on the basis of its acceptability for the purpose at hand. There are many obscure phenomena which exceed in sensitivity the more conventional tests, by several orders of magnitude. Thus, many years ago, Harvey found that certain microorganisms, in the presence of the appropriate enzyme, would luminesce in the presence of oxygen. By generating the latter at microelectrodes and measuring the electrolyzing current, such luminescence could be detected for oxygen concentrations corresponding nearly to individual molecules.

Another approach to the purity question is to establish the degree of homogeneity of a sample. For this purpose chromatography has furnished a most powerful tool. To be sure, one must use several adsorbents and a variety of developing solvents before concluding that the sample is not resolvable into more than one component. Nevertheless, there are numerous examples in the literature of chromatography, in which a substance judged to be "pure" and homogeneous, was readily resolved on a column into several distinct components. Just as we speak of a spectrographically pure metal specimen, it has become increasingly customary to speak of a material as being chromatographically homogeneous.

It seems to us that differential instrumental methods of examination can contribute increasingly to purity criteria and, as always, this assumes that small amounts of a very pure sample are available for comparison. Aside from the more commonly employed spectrophotometric methods, in all regions of the spectrum, it would be profitable to develop and improve differential techniques in refractometry and dispersion and the techniques for differential thermal history.

Characterization

In characterization studies to determine the identity of a substance, purity enters as a secondary consideration, usually one to be taken up later. The two questions cannot always be considered separately, because if some of the simpler criteria such as melting point, boiling point, or other colligative properties are used, impurities will lead to erroneous or misleading answers. More distinctive criteria such as x-ray and electron diffraction, or spectrophotometry, will indicate and identify the bulk constituent and frequently afford an indication of the nature and amount of the impurity. For all but the simplest molecules, the information obtained by these methods is empirical and will, no doubt, remain so for a long time. In the infrared field alone, one of the main problems has been to find an economical method of storing vast amounts of empirical information in a form readily available for reference.

Electronic Analog System

We have thought for a long time that a synthetic approach to this question is

INSTRUMENTATION

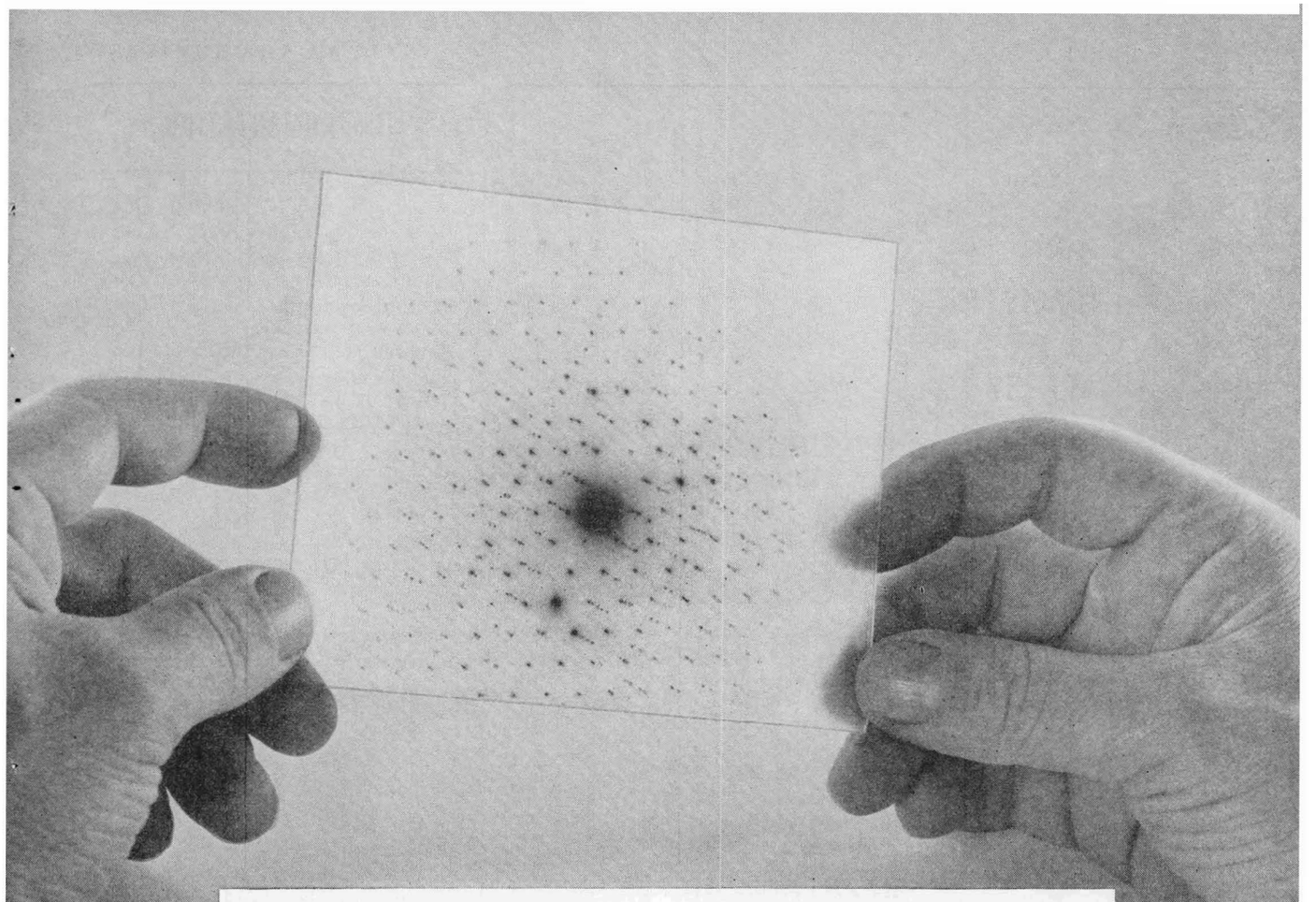
worthy of investigation. One recalls the considerable success which attended Andrews' early attempts to predict Raman frequencies by means of dynamic molecular models. In these studies, models were constructed of spheres, the masses of which stood in the ratio of the respective atomic masses, and these were coupled with springs, the elastic constants of which were carefully measured. When such a loose aggregate or model was suspended and then "jiggled" by a variable speed motor, a varied set of motions of the model resulted. The model was illuminated stroboscopically and, at stated speeds of agitation, characteristic resonant periods became evident. There were many of these and it was possible to correlate these mechanical frequencies with the Raman frequencies for the molecule by direct factors relating model "atom" mass to one actual atom and the spring constants to interatomic binding forces.

From our point of view, it might be instructive to set up the electronic analog of such a system. Imagine that one were to set up a series of electron-tube oscillators, the frequency of each of which was a definite submultiple of a characteristic bond frequency, corresponding to a C—C, C≡N, C=O, etc. Through suitable buffer amplifiers, the amplitude of these characteristic frequencies could be varied over wide limits. The several frequencies could be combined in a mixer stage and perturbation effects could be simulated very easily by standard frequency-modulation techniques. Any desired forcing function could be applied to this system and the complex output could be viewed on a cathode-ray oscillograph. It is obvious that an infinite number of bewildering patterns could be produced in this manner, but the real utility would seem to lie in the following possibility. If one could take a complex infrared recording and scan it optically for cathode ray oscillograph presentation, then one would manipulate the electronic analog device until it produced the same pattern. From the necessary settings one would read off frequencies, amplitudes, and coupling factors from which a probable molecular configuration could be deduced.

Complexity of Problem

We should report that the few experts on molecular structure and infrared spectroscopy, with whom we have discussed these possibilities, have taken a dim view of the idea. It is possible that they are not fully aware of the capabilities of electronic analog systems, but

(*Continued on page 26 A*)



The electrons landed here...

. . . and the diffraction pattern they left on this plate measures the splitting of the laminae in a 1000-Angstrom-unit-thick sheet of bent mica through which they passed.

For industry, electron diffraction patterns are revealing and identifying oxides on non-ferrous metals, the faint beginnings of corrosion on steel, scale in distillation equipment, crystal formations in chemical processes—thin films of all kinds.

A host of fine instruments that are serving science and industry today are built around the simplicity, the economy, the easy reproduction, and the incontrovertibility of photographic recording. We make the specialized sensitive materials they require—for electron diffraction and electron micrography, for spectrochemistry, for x-ray diffraction, for mass spectrography, for work in the infrared and the deep ultraviolet, for autoradiography with radioisotopes, for capturing tracks of cosmic ray primaries in the upper atmosphere and mesons in the great nuclear accelerators, for analyzing the stuff that distant galaxies are made of.

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INSTRUMENTATION

more likely that we have not fully appreciated the complexity of the problem. This much may be said for the possibilities of harmonic synthesis by electronic systems. Even with oscillators exhibiting linear performance, it requires relatively few of them, even without intercoupling, to produce complex patterns. The additional possibility exists of operating them in nonlinear fashion as individual harmonic generators. It may be of interest to note that Pepinsky has made notable progress in the crystallographic field by a related approach in which Patterson diagrams can be synthesized and presented on an oscillograph.

Andrade was not altogether facetious when he once remarked that "the elucidation of atomic structure by measuring the light which an atom emits is much like asking an individual who has never seen a piano to envision its size, shape, and appearance by listening to the sound which it emits when it is thrown down a flight of stairs." Physical analogs and models have been very useful on occasion. While it is true that the behavior of very simple polyatomic molecules can be described accurately in quantum mechanical terms, this is out of the question for a thing like penicillin. For one thing, the computational difficulties increase prohibitively as molecular complexity increases. One wonders, however, to what extent high speed electronic computers may provide some solution by a straight forward, albeit lengthy computational process. Then too, the stochastic approach, in which a huge number of shrewd guesses are made at high speed, has its possibilities. Just what the criterion of the correct guess would be is probably the most difficult question.

It seems to us that there is a considerable need for more inquiry into the empirical relations which might be found for infrared data. It is evident that empiricism will be necessary for some time to come, but almost any generalization would be very useful. The industrial analyst has a full day's work before him and continues to consult his punched card index, record his findings, and dash on to the next chore. The academic investigator may spend months or years in unraveling structural details of a single molecule. In the interim, the analyst's general requirements are not met, because he can decide, with certainty, on a structure only if it previously has been recorded.

Instrumentation continues to improve the techniques used in infrared studsie, but it may well be called upon to solve some of these more general questions.

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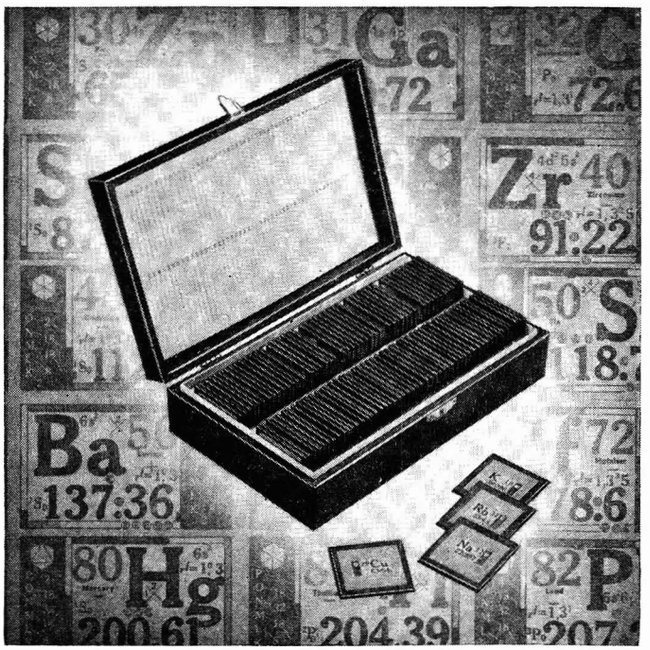

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NEW PRODUCTS FOR ANALYSTS

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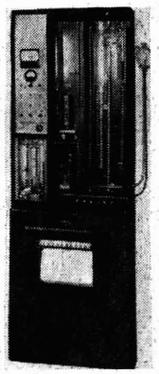
Adsorption Fractionator

A new method of adsorption fractionation is the result of more than 10 years of research and experimentation in the Burrell Corp. laboratories. The method permits the rapid and accurate analysis of light hydrocarbon gases, through and including pentanes. The gases are separated, identified, and measured by volume of separated fractions. Separation is achieved by physical adsorption. Identification is made by measurement of thermal conductivities, and the volume of each fraction is arrived at by determination of the change in pressure of an evacuated system. Accuracies of the order of 0.2% are obtained. Identifications and measurements of the gas fractions are recorded on a potentiometer, so that a complete and automatic record of each analysis is available to the operator.



Contrasted with previously available methods requiring from 6 to 8 hours, the new fractionator does the job in from 1 to 3 hours with approximately 300 ml. of sample. Gases containing predominantly light fractions, which have presented difficulty with other methods, may be analyzed reliably. Operators need not be skilled. The Burrell apparatus is relatively low in cost. No liquid air or other refrigerant is needed. An electrical connection is the only requirement for immediate operation. The instrument measures 2 × 3 × 6 feet. **1**

Labeling Tape

A pressure-sensitive labeling tape is now available in four colors and four widths from the Labelon Tape Co. Originally introduced in two colors and two widths, the tape is currently produced in black, blue, red, and green in 0.625-, 0.75-, 1.0-, and 1.5-inch widths and comes in 400-inch standard and 800-inch industrial rolls. Made of two layers of acetate with a white waxy substance laminated in between, the tape derives its writing qualities from the fact that the pressure of the pencil or stylus, rather than the lead, makes the impression on the tape. Pressure on the top clear layer removes the white waxy substance and exposes the bottom colored layer to view, in a manner similar to that of a child's "magic slate" toy.

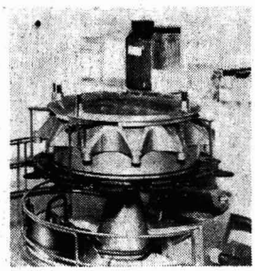
The neoprene-base adhesive, which requires no moistening, adheres readily to wood, glass, plastics, metals, and ceramics. The tape may be transferred from one surface to another repeatedly without leaving a sticky residue or destroying the adhesive qualities of the tape. Made of nonhygroscopic acetate, the product will not turn yellow with age, discolor, or curl at the edges. **2**

Bismuth Wire and Ribbon

Fitzpatrick Electric Supply Co. has announced the successful production of ductile bismuth wire and ribbon. While all the electrical and physical properties of bismuth were known at the turn of the century, no practical use for bismuth was developed because there was no known way to overcome its brittleness and lack of tensile strength. The company claims to have surmounted these difficulties to a large extent. It has produced bismuth that is ductile enough to be wound on its own diameter at room temperature. However, its tensile strength is still relatively low and consequently the material must be handled with care. **3**

Blood Sugar Determinations

The precise determination of blood sugar content within limits of a diagnostic range in less than 5 minutes is now possible with a new machine developed by the Mathewson Machine Works. Called the Hewson Clinitron, the apparatus has been used to run tests on 4300 blood specimens in approximately 40 hours, with only one attendant. Without the machine, the processing of this number of specimens would require 10 to 15 weeks of a laboratory technician's time, with a labor cost alone of several hundred dollars.



A specially formed General Electric tubular heater permits closely controlled heating of the test tubes at various steps in the process. Tablets of zinc hydroxide, potassium iodide,

Circle desired items for further information on items listed in this section

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AUGUST 1951

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potassium ferricyanide, and tartaric acid are added to the specimen during the reaction. The test tubes are progressively heated, steamed, and subjected to the subdued heat of the tubular heater. The test tubes are then tilted, shaken, and cooled. If the blood sugar value is below the screening level, the solution will be blue. If above this level, indicating an increase of blood sugar above the standard range, the solution will be colorless. This affords a precise, easy reading by the unassisted and untrained eye.

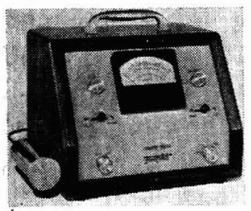
The machine is portable, weighing 53 pounds. The heating element is protected by a guard, and a reflector slide provides heat adjustment in the event that the voltage drops below the normal 110 volts. **4**

N-Bromomethylphthalimide

N-Bromomethylphthalimide is the latest in a series of reagents announced by the Dajac Laboratories. The compound offers advantages over the more commonly used reagents for synthesizing derivatives of alcohols and phenols. It is stable and can be stored without special precautions. Solid derivatives may be formed even with low-molecular-weight alcohols. The reaction is rapid and the derivatives may be easily separated and purified. The chance of undesirable side products being formed is eliminated. A table of the phthalimidomethylene ethers that have already been characterized is available. **5**

Radiation Monitor

The new instrument, SU-3B, offered by Tracerlab, Inc., has been developed for use as a routine contamination monitor in radioactivity laboratories.

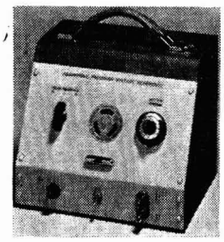


It may be employed in checking laboratory bench tops for contamination (including contamination from such low-energy beta emitters as C^{14} and S^{35}), checking glassware in tracer experiments for adequate decontamination, monitoring "background" counting rate to detect large-scale fluctuations, and monitoring hands and laboratory coats for contamination. This compact, direct-reading, a.c.–operated instrument has three full-scale meter ranges of 200, 2000, and 20,000 counts per minute. It comes completely equipped, including a mica end-window Geiger tube enclosed in a detachable probe assembly and connected to the instrument by a convenient length of flexible cable.

The laboratory monitor weighs 17 pounds, is housed in a walnut cabinet, and has a clip for holding the Geiger tube horizontally near the table top. Other features include a built-in loud speaker monitor at the rear of the chassis for use as an aural monitor, a control on the front panel for adjustment of the volume, and a pulse-generating circuit, synchronized with the 60-cycle line frequency, which allows a quick calibration check to be made without the use of an external generator. The monitor is capable of driving a recorder having low current drain. **6**

Direct Current Source

A new all-electronic instrument which operates from a.c. lines to provide a continuously variable d.c. supply over the range of 0.0001 to 10 volts has been developed by the General Precision Laboratory. The voltage source is a precision unit designed primarily for use in connection with high impedance devices—for d.c. amplifier testing, for the calibration of d.c. oscilloscopes and vacuum tube voltmeters, and for other laboratory and plant uses.



The maximum output impedance of the unit is 1000 ohms, with accuracy maintained at 0.1% of full scale. A multiple-turn potentiometer is provided having divisions of 0.001 of full scale. The circuit may be operated with input voltages of 105 to 130 volts at 50 to 60 cycles. The unit is housed in a metal cabinet 10 inches wide, 8 inches deep, and 8.75 inches high. Fuse, output terminals, and controls are placed in front for easy accessibility. **7**

2,4-Dinitrofluorobenzene

The compound 2,4-dinitrofluorobenzene, now available from Jasonols Chemical Corp., is a pale yellow liquid having a melting point of 22° C. and a characteristic pungent odor. It has found use as a fluorinating agent and, more recently, as a reagent for characterizing alcohols and other compounds containing labile hydrogen atoms. The increased activity of the fluorine in dinitrofluorobenzene is caused by the two nitro groups and the resonance structure of the quinoid grouping. The reaction of dinitrofluorobenzene with alcohols produces an ether and, thus far, several 2,4-dinitrophenyl ethers have been isolated. **8**

Washfastness Testing

Atlas Electric Devices Co., manufacturer of Launder-Ometers, Fade-Ometers, and Weather-Ometers, has developed new specimen containers for the No. 3A tentative accelerated

washfastness test recently adopted by the AATCC. This new test, employing metal specimen containers 3.5 inches in diameter and 8 inches in length, duplicates the color destruction and abrasive action of five average commercial or home launderings in a single 45-minute test. This is one tenth of the time required by the old test methods.

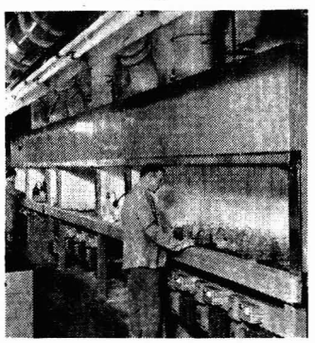
Through the use of easily installed adapters, the unbreakable containers are mounted horizontally at right angles to the shaft in any standard or research Launder-Ometer. Mounted in this fashion, the test specimens are subjected to a forceful throw, a long slide, and a strong impact. The 100 steel balls in each container and a lower liquor volume also increase the abrasive action. The tentative test is applicable to cotton or linen textiles in fabric form and is especially designed for evaluating the washfastness of colored textiles expected to withstand frequent laundering. As many as 20 samples may be tested simultaneously. **9**

Magnetic Stirrer

Manufactured by Laboratory Industries, Inc., the Magne-Stir, a new magnetic stirrer, employs Teflon-covered stirring magnets which are resistant to breakage and to attack by acids and alkalies. The motor will stir 1 liter or more of liquids up to 60% glycerol solution at a speed of over 1800 r.p.m. A separate on-off switch permits the stirrer to be turned on or off without change in the speed setting. The cast aluminum housing is designed either for use directly on a bench or table or mounted securely on a setup frame. The stirring bars, covered with a 0.031-inch layer of Teflon, are available in three sizes: 0.31 × 0.75, 0.31 × 1.25, and 0.31 × 1.75 inches. Stirring bars covered with borosilicate glass can also be furnished in several sizes. Magnetic stirrer is available for voltages of 115 and 230 volts at 50 to 60 cycles. **10**

Glass-Lined Hood

Glass-lined fume hood, with a water-spray system in the exhaust ducting, minimizes the danger of acid fumes in the chemical analysis laboratory of Pratt & Whitney Aircraft. The new unit, designed and installed by Pratt & Whitney, points the way to similar safety advances in other laboratories. The danger arises in the company's laboratory from the absorption of fumes in the lining of the hoods where analytical reactions on metal test specimens are carried out. A hot bath of perchloric acid, which is used in a great many of these analyses, generates the fumes and causes deposits to accumulate on the inner surfaces of the exhaust piping system, as well as in the joints and behind the lining of the test hoods.

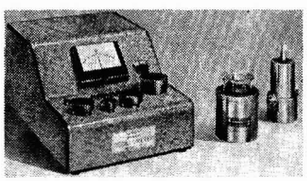


Efforts to deal with the deposits by a regular program of cleaning, which involved washing the hood lining and scraping off the accumulated rust and deposit in the exhaust piping, proved unsatisfactory. These difficulties were eliminated by the design of the new hood. The installation is a six-compartment unit having a total length of 36 feet and a height of 7.25 feet. Each recess is lined with sheets of white Carrarra structural glass, 0.344 inch thick on the walls and ceilings and 0.75 inch thick on the working surface top. Carrarra glass, a Pittsburgh Plate Glass Co. product, will not absorb the fumes or deposits. It is kept clean simply by wiping with a wet paper towel or cloth. The glass is shock-resistant and withstands the range of temperatures encountered in the laboratory. Joints in the glass lining are made of silicone rubber, which by its resiliency provides a satisfactory seal and cushioning effect. Moreover, it does not react with perchloric acid to form highly flammable compounds, as do other carbon-base rubbers. For further protection, the rubber is wrapped in Teflon, which is even more highly resistant to chemical attack.

A key to successful performance is the special water spray equipment in the exhaust piping system. This spray operates continuously when the exhaust blower is running. The spray serves as a continuous wash-down system for the exhaust piping, and not only keep it clean and free from the accumulation of dust and deposit but also protects the inside of the pipe from corrosive attack. The hood installation represents a safety investment of $15,000. **11**

Oscillometer

E. H. Sargent & Co. has announced the availability of a new high-frequency oscillometer of the resonance-retune type. In principle, the increase in the capacitance of a sample cell containing the unknown is balanced by the removal of a fixed capacitance, which is adjusted by a range switch and by the setting of a high-precision variable condenser. This condenser has a precision of approximately ±0.005 mmf. over a range of 5 mmf., or with departures from linearity of the order of 0.05%. The instrument will accommodate a capacitance change of from 0 to 50 mmf. Appropriate cells permit the titration of electrolytes, as well as the measurement of small samples or flowing systems. The instrument is sensitive to traces of polar materials in nonpolar subtances and may be used to determine the composition of mixtures containing organic compounds. The oscillometer may also be used in acidimetric and precipitation determinations. **12**



Flexible Manometer

A manometer offered by F. W. Dwyer Manufacturing Co. can be rolled into a shape that can readily be fitted into a pocket. The manometer, made of flexible vinyl plastic, can be rolled, twisted, or bent into any form. The U-tube will return to its full length when unrolled. The adjustable plastic scale is firmly held between the tubes. **13**

Freeze-Drying Units

A compact, factory-assembled freeze-drying unit, Model 203F, has been announced by the F. J. Stokes Machine Co. It employs Freon refrigeration for the drying of guinea pig complement, cultures, serums, vitamins, and other biologicals. Drying and freezing are accomplished in a tank adjacent to the condensing chamber, on an electrically heated and thermostatically controlled drying shelf. The sight glass in the lid permits inspection during freezing. The unit has a batch capacity of 3500 ml.

Model 203 freeze-drying unit has a batch capacity of 900 ml. Four complete drying cycles may be made before regeneration of the desiccant is necessary. Standard equipment includes a vacuum pump and McLeod gage, in addition to nine desiccant baskets and a supply of desiccant. **14**

McLeod Gage

A new McLeod gage, suitable for the ASTM proposed test for the distillation of petroleum products at reduced pressures, is now being distributed by the Emil Greiner Co. The instrument has scales graduated in both the square and linear systems, the former permitting more sensitive observations. Readings are made directly in millimeter values. Individual

calibrations of volume ratios can also be made directly. The gage possesses various desirable features, such as a trap to prevent the overflow of mercury, a heavy-walled mercury reservoir, a specially designed air filter that prevents contamination of the system, and a constriction of the main tube that prevents breakage of the flask by a sudden return of the mercury. The instrument has a range from 0.1 to 20 mm. linear and 0.01 to 20 mm. square. The manufacturer also offers a series of other McLeod gages which allow maximum sensitivity in the various working ranges desired. **15**

Odor Control

A tiny lamp capable of dissipating odors has been developed by engineers of the Westinghouse Lamp Division. Three times as powerful as the ozone lamp introduced in 1945, the new Odorout bulb emits ultraviolet radiations which transform the oxygen around the lamp into ozone, an air purifier. The 3.5-watt, walnut-sized lamp, when burned in a special wall fixture, destroys cooking, smoking, dampness, mildew, and other odors. The bulb alone sells for $1.30 and lasts 6 months when operated 24 hours a day. It must be burned in a special fixture with a current-controlling device such as a transformer. Eight manufacturers now produce special Odorout wall fixtures which use one to four lamps in each unit. These units range in list price from $6.95 to $18.95, including lamp. **16**

MANUFACTURERS' LITERATURE

Glossmeter and Reflectometer. An 8-page publication describes improved portable glossmeter and 45° 0° reflectometer. New optical design minimizes calibration failures. Experimental 20° unit for high-gloss surfaces and a 45° unit for ceramic materials are available. Henry A. Gardner Laboratory, Inc. **17**

Hydrogen Peroxide. Attractive 36-page brochure gives information on hydrogen peroxide: uses, handling, storage, shipment, freezing point and melting point curves, density of solutions, packaging and labeling, and factors influencing stability of compound. Details are given on the preparation and analysis of hydrogen peroxide solutions. Pennsylvania Salt Manufacturing Co. **18**

Recorder. Bulletin 330 describes and illustrates new Model MD-2 recorder, a portable, self-contained instrument which will plot automatically on rectangular coordinates any two variables capable of actuating the movable cores of miniature variable transformers, either directly or through accessory Bourdon tube units. Baldwin-Lima-Hamilton Corp. **19**

Hydrogen Ion Concentration. "The Meaning, Application, and Measurement of pH" is the title of leaflet which specifies the pH values of common acids and alkalies, the intensity of acidity or alkalinity, and the most desirable pH range of industrial waters for different conditions and applications. Allis-Chalmers Manufacturing Co. **20**

Potentiometer Controller. A 2-page announcement gives specifications for new strip-chart pneumatic control potentiometer. The controller provides a full 11-inch calibrated chart width and over 120 feet of chart length. Minneapolis-Honeywell Regulator Co. **21**

Insecticides. A 24-page booklet on toxaphene agricultural insecticides contains sections on economically important insect pests, a brief history of the development of toxaphene, and a list of common and scientific names of the insects mentioned. State and federal recommendations are given for the control of cotton insects, livestock pests, alfalfa insects, cutworms, grasshoppers, and a variety of other insect pests controlled by toxaphene. Hercules Powder Co. **22**

Riboflavin. Booklet describes use of riboflavin as an ingredient in livestock, poultry, and other animal feeds. A summary of the latest available information on the role of riboflavin in nutrition is included, as well as sections describing sources of riboflavin, deficiency symptoms, and recommended nutrient allowances. Commercial Solvents Corp. **23**

Dehumidification. Leaflet on dehumidification contains table of recommended humidities in various industries and provides information on equipment available for controlling and measuring humidity. Abbeon Supply Co. **24**

Butyl Stearate. Data sheets describe physical properties and applications of butyl stearate, a colorless, relatively nonvolatile liquid which solidifies at temperatures below 66° F. and is compatible with a wide variety of organic materials. Witco Chemical Co. **25**

Soybean Lecithin. An 18-page booklet provides information on properties and suggested uses of Arlecin, a soybean lecithin. Bulletin includes results of consistency and settling tests in which paints containing product are compared with those employing aluminum and zinc stearate as antisettling agents. Archer-Daniels-Midland Co. **26**

Storage Batteries. Brochure discusses rechargeable storage batteries which are up to one fifth the size and one sixth the weight of average storage batteries. Physical and electrical characteristics, as well as style of terminals, are indicated. Yardney Electric Corp. **27**

Combustion Tube Furnaces. A 4-page pamphlet describes combustion tube furnaces which have a maximum safe working temperature of 1000° C. For intermittent periods of comparatively short duration, the furnaces may be operated at a maximum temperature of 1065° C. Hinged-type furnaces will reach 1000° C. in 40 to 80 minutes and the solid-type furnaces in 45 to 75 minutes. Hevi Duty Electric Co. **28**

Portable Pyrometers. Two portable pyrometer models are described in Bulletin PPY-1, which also gives specifications for thermocouple holders and tips. Model 4 may be used within 0° to 3000° F. range. Wheelco Instruments Co. **29**

Oxygen Recorder. A 16-page booklet explains how an analysis is made by use of the paramagnetic properties of oxygen. Booklet contains a diagrammatic explanation of the operation of the recording section and various gas-sampling systems used with the instrument. Such features as the voltage regulator, alarm contacts, slide-wire, and cam unit are discussed. The Hays Corp. **30**

Instruments and Accessories. U-type, well-type, and multiple tube manometers, inclinometers, draft gages, liquid level gages, sight feed bubblers, and manometer accessories are discussed and illustrated in 44-page booklet. Among the new instruments described are packaged test units for use in industrial calibration work. Meriam Instrument Co. **31**

Turbidity Measurements. Pamphlet describes turbidimeter used in sulfate determinations and in measurement of suspended matter and colloids generally. Instrument may be adapted to many analytical tests commonly performed with a nephelometer. Hellige, Inc. **32**

Physical Measurements. Booklet contains descriptions and specifications of transformer, recording and dial-indicating thermometers, and recording pressure and vacuum gages. Electric Auto-Lite Co. **33**

Mercury Cleaning. A 4-page leaflet provides information on 5-, 25-, and 150-pound-capacity mercury cleaning units and gold-adhesion filters. Bethlehem Apparatus Co. **34**

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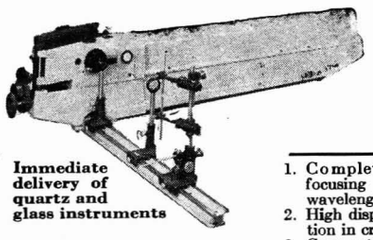

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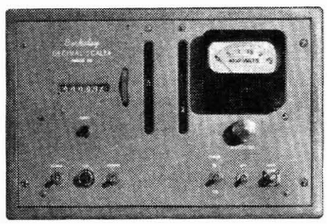

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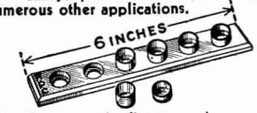

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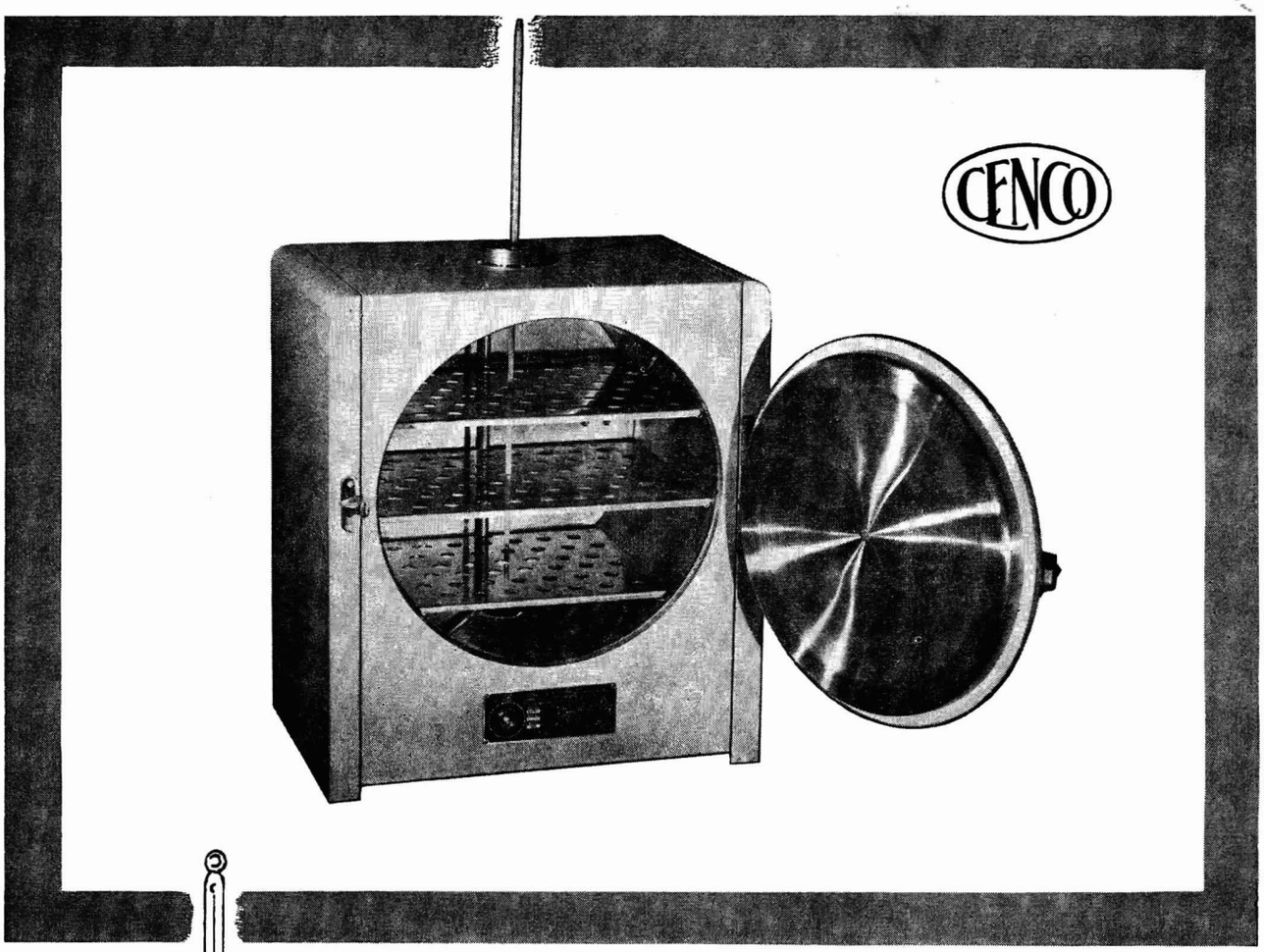

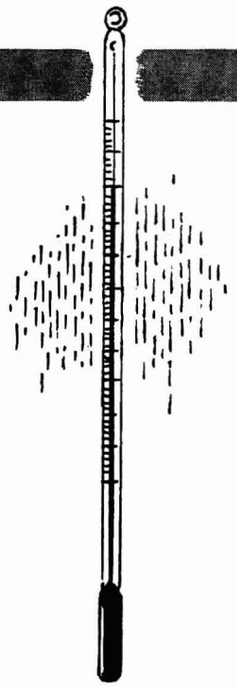

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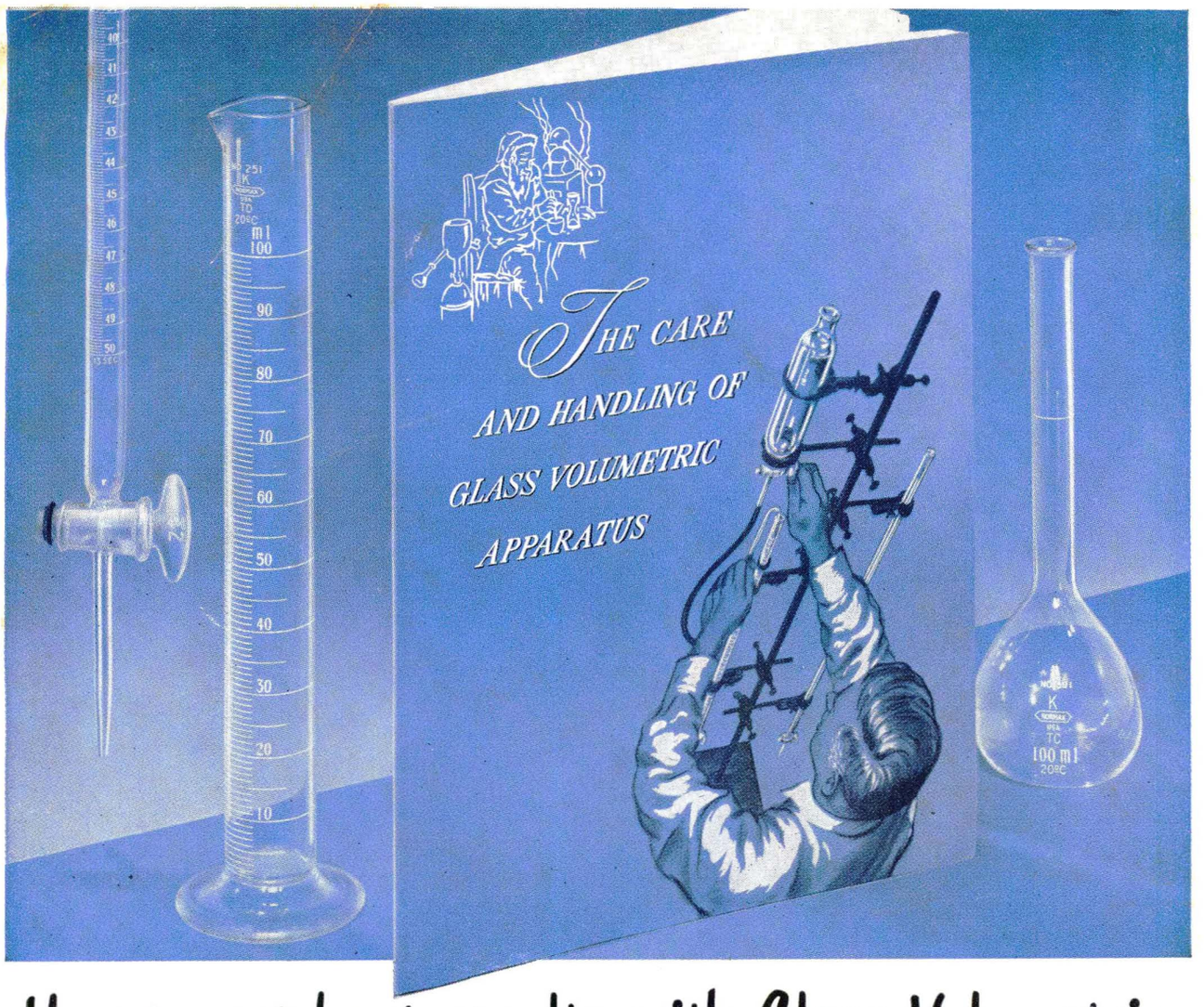

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