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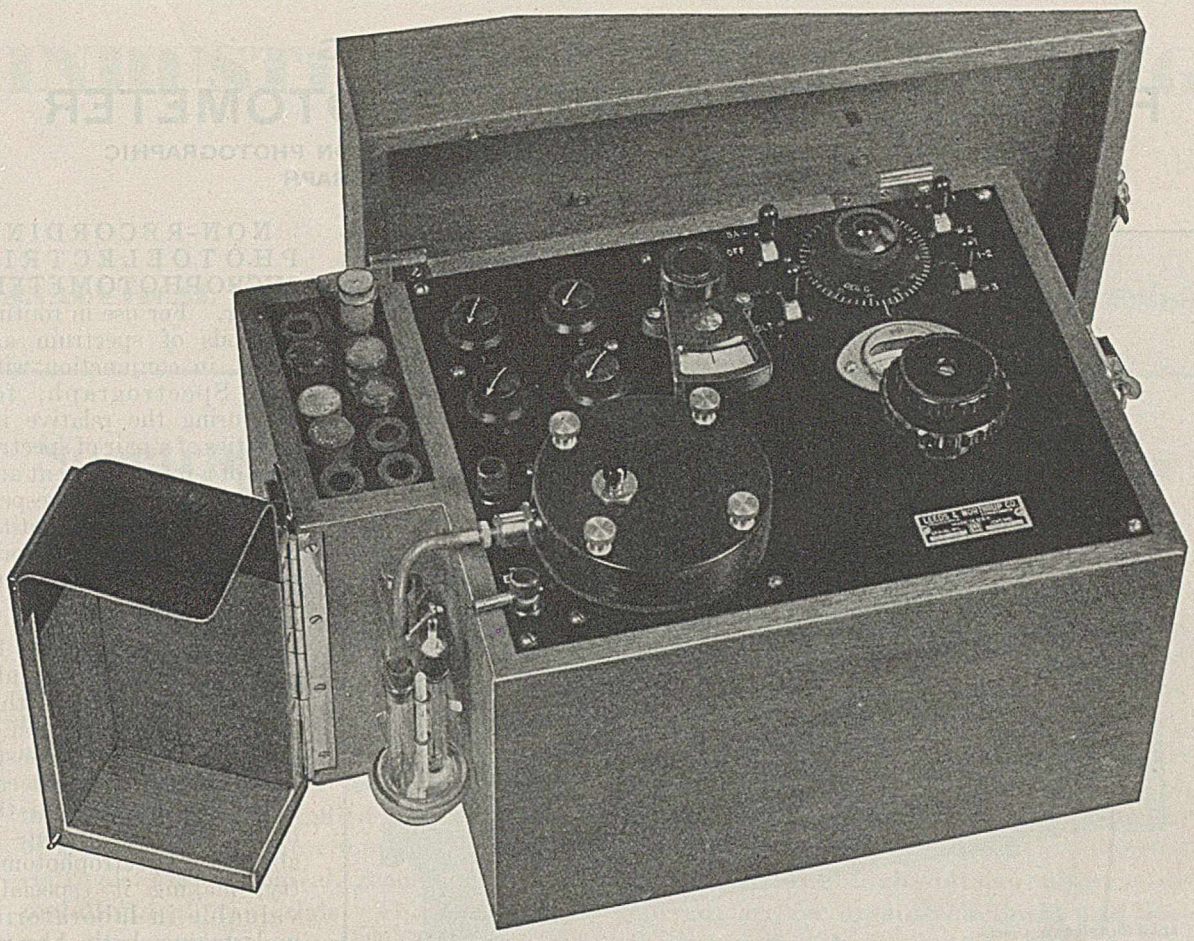

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INDUSTRIAL and ENGINEERING CHEMISTRY

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Spectrographic Methods of Trace Analysis

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The field of application of spectrographic methods for the qualitative and quantitative analyses of materials for traces of metals and metalloids has been considerably enlarged in recent years by the development of improved technique. These methods have been applied to the analyses of heavy and organic chemicals, pharmaceuticals, and biological, geochemical, and metallurgical materials. The sensitivity and accuracy of these methods have been increased by the use of spectral sources of excitation particularly adapted to the analyses of different materials and by the use of well-tested means of photographic photometry. The following sources have been found to be appropriate for the indicated analyses: the high-voltage, alternating current arc for inorganic chemical products; the direct current arc for metallurgical specimens; the cathode layer of the direct current carbon arc for geochemical samples; the direct current condensed spark and the high-voltage, alternating current arc for organic chemical and biological materials.

The speed and adaptability of spectrographic methods have contributed materially to their usefulness for research and control analyses.

SPECTROGRAPHIC analysis of materials is based upon the fact that each chemical element in the vapor state, under suitable thermal or electrical excitation, emits radiation composed of characteristic wave lengths, or spectral lines. The wave lengths of the spectral lines emitted by each element are different from those emitted by any other element. This is the basis of qualitative analysis. The intensities of the spectral lines emitted by each element under controlled conditions of excitation are proportional to the concentration of that element in the specimen. This is the basis of quantitative analysis. The best quantitative analytical technique rests upon the experimental determination of the relationship between the concentration of a constituent of a specimen and the relative intensity of a pair of selected spectral lines, one of that constituent and the other of an internal standard element present in or introduced into the specimen in constant amount.

The emission spectrum, to which this paper is limited, is suitable for the detection and determination of the metallic and metalloid elements.

For the purposes of this paper a trace element will be defined as one contained in a concentration of less than 0.01 per cent in a specimen.

This method of analysis is particularly suitable for the determinations of elements present in trace amounts. Its principal advantages are:

1. The amount of sample required is extremely small; a few milligrams suffice in many cases for a complete quantitative analysis for the metallic constituents.
2. A minimum amount of chemical preparation of the sample for analysis is required. The simultaneous identification of the different elements, and the determination of their concentrations, may be made without previous chemical separations.
3. The sensitivity is very great. Most elements can be determined in concentrations down to 0.0001 or 0.001 per cent. In some matrices certain elements may be determined down to concentrations approaching 0.000001 per cent.
4. The precision and accuracy of analysis for elements occurring in concentrations of a few ten-thousandths to a few thousandths per cent are valuable, for in many instances this method provides the only practicable means of determination.
5. The rapidity of the method, where applicable, in general saves a considerable portion of the time and cost required for a chemical analysis.
6. A complete qualitative analysis of the specimen for its metallic constituents may ordinarily be made by inspection of the same spectrum which is used for the quantitative determination of one or more elements.
7. By the use of best technique the analysis depends only upon direct measurements with instruments and at no stage upon the judgment of the analyst. The determinations made by an intelligent laboratory assistant are as reliable as those made by the spectroscopist who developed the method.

Articles printed on pages 59 to 88, inclusive, were presented at the Symposium on Recent Advances in Methods for the Determination of Traces.

Field of Application

The foundation of this method for qualitative analysis was laid by Bunsen and Kirchhoff in 1860–1861 and for quantitative analysis by Hartley in 1882. However, the method had little practical application for many years because the procedures employed were not readily reproducible and the quantitative results were not sufficiently accurate. In recent years the field of application of this method has been considerably enlarged and its practical success ensured by the development of improved technique. The sensitivity, accuracy, and speed have been greatly increased by the use of spectral sources of excitation particularly adapted to the analyses of different types of materials and by the use of well-tested means of measuring the intensities of spectral lines.

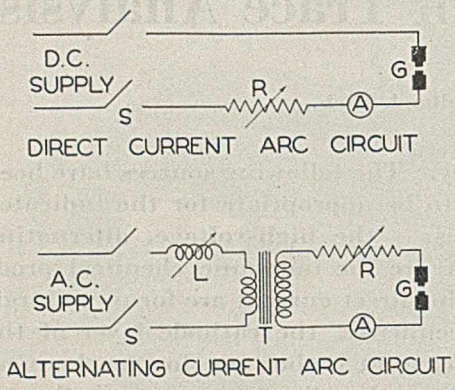

FIGURE 1. CIRCUIT DIAGRAMS

Direct current arc and high-voltage alternating current arc sources

S.	Line switch	*R.*	Variable resistance
L.	Variable inductance	*A.*	Ammeter
T.	Transformer	*G.*	Analytical arc gap

The applications of the method now include the analyses of practically any solid, liquid, or powdered material containing metallic or metalloid constituents or impurities. Its advantages have led to its regular use for quantitative control analyses of several commercial products, including metals, alloys, and heavy and organic chemicals. In addition, it has proved valuable for quantitative trace analyses of biological, agricultural, and geochemical specimens.

Experimental Technique

EXCITATION OF SPECTRA. The chief factor in the sensitivity of spectrographic analysis is the type of spectral excitation employed. The sensitivity, as well as the analytical accuracy, has been considerably increased by the use of spectral sources particularly adapted to the analyses of different materials. The following sources have been found to be appropriate for the analyses of the indicated types of materials for small amounts of impurities:

Inorganic chemical products: the high-voltage, alternating current arc
Metallurgical specimens: the direct current arc
Geochemical samples: the cathode layer of the direct current carbon arc
Organic chemical and biological materials: the direct current condensed spark and the high-voltage, alternating current arc

These classifications are not rigid, but indicate the most favorable sources as found in practice.

Direct Current Arc. The wiring diagrams of the direct current and of the high-voltage, alternating current arcs are shown in Figure 1.

The direct current arc is maintained between two electrodes of an electrically conducting, solid sample, or between two graphite or metallic electrodes in a cavity of one of which a small amount of a powdered or liquid sample is placed. Arc currents of from 1 to 15 amperes are ordinarily used. The sensitivity of detection of impurities usually increases with the current on account of the higher temperature attained.

Cathode Layer of Direct Current Carbon Arc (12). In contrast with usual direct current arc practice, in this source the sample material is placed upon the cathode. This source utilizes the experimental fact that in a region 1 to 2 mm. from the cathode the intensities of the spectral lines of most metals are enhanced from 5- to 100-fold over their intensities in the positive column of the arc. This enhancement is produced by an increased concentration of metallic atoms in that region which is caused by the ionization of vaporized atoms in the arc gas, the migration of these ions to the cathode, and their neutralization there. This enhancement is most pronounced for small amounts (1 to 3 mg.) of sample and is decreased by adding to the sample a large amount of a substance of lower ionization potential than the test element. This source has proved particularly valuable for the analysis of nonconducting geochemical samples for minute traces of impurities (*16*).

High-Voltage, Alternating Current Arc (5, 14). Arc currents of from 1 to 6 amperes are ordinarily used at potentials of 1,100 or 2,200 volts. The arc may be maintained between solid electrodes of the sample or between two graphite or metallic electrodes upon each of which a drop of the test solution has been dried.

This source has been found to be extremely important for trace analyses of chemical materials. Its chief advantages include reproducibility of excitation conditions, high sensitivity, low background density, and small amount of sample required.

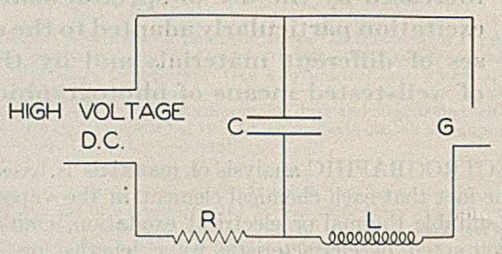

FIGURE 2. CIRCUIT DIAGRAM

Direct current condensed spark source

R.	High resistance	*L.*	Inductance
C.	Capacitance	*G.*	Analytical spark gap

Direct Current Condensed Spark (10). While the high-voltage, alternating current condensed spark is advantageous for the analyses of metallic alloys for constituents in amounts greater than 0.1 per cent, the low-voltage, direct current condensed spark is suitable for trace analyses of biological materials. A wiring diagram of the latter spark circuit is shown in Figure 2. As used, the spark takes place between a plane metal electrode upon which the sample is spread and a pointed metal electrode.

The advantages of this source include high sensitivity, concentration of the radiation in the arc spectrum, low background intensity, and no ashing and only minimum amount of chemical preparation of the small amount of sample required.

Summary of Spectral Sources. The direct current arc is suitable for the analysis of material in the solid, powdered, or liquid state. While the alternating current arc and the direct current condensed spark may be used directly for the analysis of solid electrodes, they are best suited, with present

usage, for the analysis of solutions which have been evaporated on the electrodes.

The average absolute sensitivities, in terms of the amount of metallic element determinable on the electrode, of the sources described are:

Direct current arc	10^{-5} to 10^{-4} mg.
Cathode layer of direct current carbon arc	10^{-6} to 10^{-5} mg.
High-voltage, alternating current arc	10^{-6} to 10^{-5} mg.
Direct current condensed spark	10^{-6} to 10^{-4} mg.

PHOTOGRAPHIC PHOTOMETRY. The development of precise methods of spectral photometry in recent years has contributed more than any other factor to the marked improvement in analytical accuracy.

Early methods for determining the concentration of a test element in a specimen were based upon various modifications of the general procedure of estimating, by visual inspection, the abundance of the element by reference to a series of standard spectra in which this element was varied over a known range.

TABLE I. ANALYSIS OF CAUSTIC LIQUORS

Test Element	Range of Analysis, 25% NaOH Solution %	Sensitivity (Element on Electrodes) Mg.
Al	0.000053–0.0074	2.5×10^{-5}
Ca	0.000039–0.0036	2.0×10^{-5}
Mg	0.00003–0.022	1.5×10^{-5}
Si	0.0005–0.05	2.5×10^{-4}
Cr	0.00002–0.01	1.0×10^{-5}
Cu	0.00001–0.005	5.0×10^{-6}
Fe	0.00001–0.01	5.0×10^{-6}
Mn	0.000002–0.00052	1.0×10^{-6}
Ni	0.000075–0.01	3.8×10^{-5}
Pb	0.00002–0.0034	1.0×10^{-5}
Sr	0.00001–0.01	5.0×10^{-6}

The analysis is now made, in best practice, by a photometric measurement of the true relative intensity of a line of the test element and of a line of a control element present in or introduced into the specimen in constant amount (*7*, *11*). This relative intensity is a measure of the concentration of the test element. The actual relationship is experimentally determined for each element by measurements made upon the spectra of a series of specimens of known composition in which the test elements vary over the desired ranges. The graph of this relationship, illustrated in Figure 3, provides an analytical curve from which future analyses are made (*15*).

Photometric technique is now sufficiently reliable to limit the error in the measurements of relative intensities to less than ±5 per cent.

Representative Trace Analyses

The following representative analyses are briefly described in order to illustrate the diversity of the successful applications of quantitative spectrographic trace analyses and to give the technique found most suitable in each case. The ranges of abundance of the test elements, as given, are not necessarily the only ranges in which the analyses may be made, but are those of practical interest. The sensitivity of determination of each element corresponds to the lower limit of the range of analysis for that element.

HEAVY CHEMICALS. A representative application in the field of heavy chemicals is the analysis of caustic liquors, especially those supplied to the rayon industry, for the metallic impurities: iron, silicon, aluminum, lead, manganese, chromium, calcium, copper, nickel, strontium, and magnesium (*6*). The spectrographic method provides the only practical means of analysis for these impurities in their usual concentration ranges.

One drop of a 25 per cent sodium hydroxide solution is evaporated on each of two purified graphite electrodes and the spectrum of the dry salt residue is excited in an alternating current arc. Only about 12 mg. of dry sodium hydroxide are used in the arc. The analysis is made from analytical curves, determined for each element by means of the relative intensity of a line of the test element and of a line of molybdenum, the internal standard introduced into each solution in constant amount. A typical analytical curve is shown in Figure 3.

The percentage ranges under analysis and the sensitivity are given in Table I. The absolute limit of detection is approximately 1×10^{-6} mg. of test element upon the electrodes.

This method is not only considerably faster but also more accurate than the corresponding chemical analysis. The average error amounts to no more than 5 to 10 per cent of the amount present.

TABLE II. ANALYSIS OF ORGANIC CHEMICALS

Test Element	Range of Analysis %	Sensitivity (Element on Electrodes) Mg.
Fe	0.0001–0.02	6×10^{-5}
Cu	0.0001–0.02	6×10^{-5}
Al	0.0001–0.01	6×10^{-5}
Ca	0.0001–0.01	6×10^{-5}
Mg	0.0001–0.01	6×10^{-5}
Mn	0.0001–0.01	6×10^{-5}
Pb	0.0001–0.01	6×10^{-5}
Si	0.0001–0.01	6×10^{-5}
Sn	0.0001–0.01	6×10^{-5}
Sr	0.0001–0.01	6×10^{-5}
Ni	0.0003–0.02	1.8×10^{-4}
Zn	0.0005–0.01	3×10^{-4}

ORGANIC CHEMICALS. Organic chemicals of various types, including plastics, are analyzed for the metallic impurities in the concentration ranges given in Table II (*4*).

The sample (0.40 gram) is prepared for analysis by digestion in mixtures of spectroscopically pure sulfuric, nitric, and perchloric acids, and to the resulting solution are added suitable internal standards and a sodium salt to serve as a spectroscopic buffer. The spectral source consists of an alternating current arc between two purified graphite electrodes upon each of which 0.03 ml. of the prepared solution

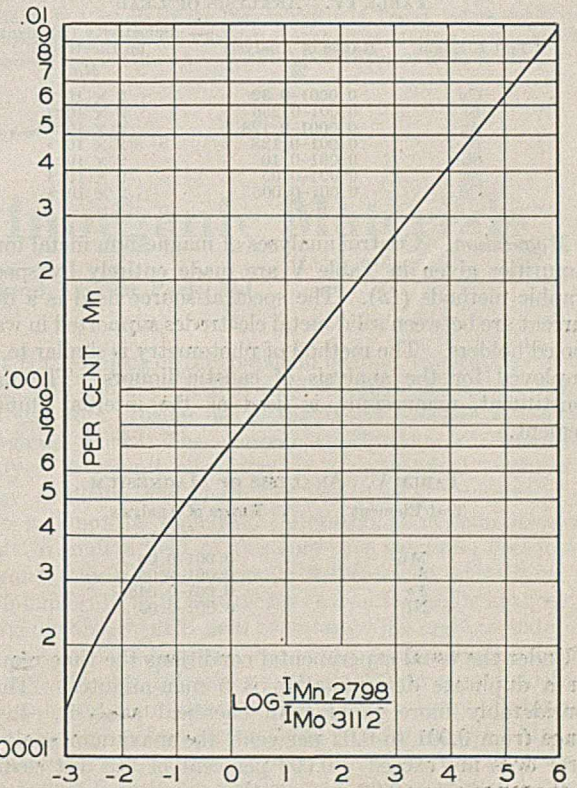

FIGURE 3. TYPICAL ANALYTICAL CURVE
Analysis of caustic soda for manganese

has been dried. The photometric method is identical with that used for the analysis of caustic liquors.

Under the usual conditions of analysis of a batch of six samples, the time required for the determination of each test element is about 5 man-minutes. The average error, obtained by repeat analyses of the same specimen, amounts to approximately 10 per cent of the amount present.

TABLE III. ANALYSIS OF ZINC

Material	Test Element	Range of Analysis %	Sensitivity (Element on Electrode) *Mg.*
Pure zinc	Pb	0.0002–0.10	1×10^{-4}
	Fe	0.0001–0.10	5×10^{-5}
	Cd	0.00005–0.10	2.5×10^{-5}
Zinc alloy die casting	Mg	0.004–0.25	1.3×10^{-3}
	Ni	0.004–1.0	1.3×10^{-3}
	Cu	0.0005–1.0	1.7×10^{-4}
	Fe	0.0005–1.0	1.7×10^{-4}
	Pb	0.0004–0.10	1.3×10^{-4}
	Cd	0.00005–0.10	1.7×10^{-5}
	Sn	0.002–0.05	6.6×10^{-4}

METALLURGICAL SPECIMENS. *Zinc.* The compositions of pure zinc and of zinc alloy die castings are regularly checked spectrographically for the impurities given in Table III (*2*).

The spectra are obtained with a 15-ampere direct current arc between graphite electrodes. The positive electrode is treated with an acid solution of the test sample. The analysis is made by visual comparison of the spectrum of the test sample with the spectra, placed upon the same plate, of standard samples of known composition with a precision of ±10 per cent of the amount present. This method will consistently detect an offgrade composition if the concentration of any test element is 20 per cent or more higher than the corresponding concentration in the standard.

Lead. The spectrographic method is employed to analyze high-grade pig lead for the impurities shown in Table IV (*1*). The technique used and the accuracy obtained are similar to those of the analysis of zinc.

TABLE IV. ANALYSIS OF LEAD

Test Element	Range of Analysis %	Sensitivity (Element on Electrode) *Mg.*
Cu	0.0001–0.32	2×10^{-5}
Bi	0.001–0.256	2×10^{-4}
Ag	0.0001–0.128	2×10^{-5}
Ni	0.001–0.128	2×10^{-4}
Sb	0.001–0.10	2×10^{-4}
Sn	0.001–0.05	2×10^{-4}
Cd	0.001–0.005	2×10^{-4}

Magnesium. Control analyses of magnesium metal for the impurities given in Table V are made entirely by spectrographic methods (*13*). The spectral source used is a direct current arc between solid metal electrodes supported in water-cooled holders. The method of photometry is similar to that employed for the analysis of caustic liquors. The major constituent, magnesium, is used as the internal standard element.

TABLE V. ANALYSIS OF MAGNESIUM

Test Element	Range of Analysis %
Mn	0.001–0.05
Si	0.001–0.05
Fe	0.001–0.045
Ni	0.001–0.05

Under the usual experimental conditions the time required for a duplicate determination is 5 man-minutes. This is considerably more rapid than chemical analysis. In the range from 0.001 to 0.02 per cent, the maximum analytical error does not exceed ±0.002 per cent of the test element. In the range above 0.02 per cent, the average error is about ±5 per cent of the amount present.

BIOLOGICAL MATERIALS. *Body Fluids and Tissues.* An interesting recent development of the use of spectrographic methods has been their application to the studies of the human body and its functions.

Methods have been developed for the determination of sodium, potassium, calcium, and magnesium in urine, blood, and saliva (*5*, *18*), and for lead in various body fluids and organic tissues (*17*). Since the concentration ranges of interest of sodium and potassium lie above 0.01 per cent, the analyses for these elements will not be considered.

The fluid or tissue is ashed and to the resulting acid solution are added suitable internal standard elements and a spectroscopic buffer. One drop of the solution is dried upon each of the graphite electrodes of an alternating current arc. The photometry is identical with that used for the analysis of caustic liquors. The range of analysis is shown in Table VI.

TABLE VI. ANALYSIS OF BODY FLUIDS AND TISSUES

Material	Test Element	Range of Analysis %	Sensitivity (Element on Electrodes) *Mg.*
Urine, blood, saliva	Mg	0.0005–0.05	2×10^{-4}
	Ca	0.002–0.10	8×10^{-4}
Body fluids, tissues	Pb	0.00001–0.01	2.8×10^{-5}
Cerebrospinal fluid	Pb	0.000001–0.002	8×10^{-7}

This method possesses the advantages of rapidity, accuracy, and the requirement of only a small sample. Ten milliliters of urine suffice for determinations of magnesium and calcium, while 2 ml. of urine or a few milligrams of skin or tissue suffice for an analysis for lead. The average analytical error is approximately 5 per cent of the amount present.

A similar method developed for the analysis of body fluids, organic tissues, and foods for lead by the use of the direct current arc and of a less precise method of photometry yields approximately the same sensitivity but about twice the error of the technique described above (*3*).

A technique has also been reported for the analysis of cerebrospinal fluid for lead, in the concentration range shown in Table VI, using a direct current condensed spark spectral source (*10*). One milliliter of material, which is neither ashed nor previously chemically treated, is sufficient for several determinations. A unique analysis is made of each specimen by a comparison of the relative intensities of spectral lines of lead and of an internal standard element before and after the addition to the sample of a known amount of lead. The analytical error is less than 15 per cent and two samples may be analyzed in 3 hours for lead in a concentration range in which chemical methods are not reliable.

TABLE VII. ANALYSIS OF PLANT ASH

Test Element	Range of Analysis (In Solution on Electrode) %	Sensitivity (Element on Electrode) *Mg.*
Ca	0.0001–0.50	1×10^{-4}
Fe	0.00005–0.20	5×10^{-5}
Mg	0.0001–0.50	1×10^{-4}
Mn	0.0001–0.50	1×10^{-4}
P	0.001–1.0	1×10^{-3}

Plant Tissue. Spectrographic analysis permits the determination of the distribution of elements, known to be necessary for proper growth and development, throughout a single plant and to some extent the following of this distribution throughout the life history of the plant.

A method has been developed, by the use of essentially the same technique as that used for the analysis of cerebrospinal fluid, which permits the analysis of as little as 200 mg. of plant tissue for boron in the range from 0.0001 to 0.001 per cent (*9*); 1×10^{-4} mg. of boron on the electrode is determinable. The analytical error rarely exceeds ±10 per cent and one hour is required per determination.

A procedure which is applicable to samples of even less than 10 mg. has also been worked out for the analysis of plant ash for the minor impurities shown in Table VII (*8*).

A 50-mg. sample (if available) of ash is treated with hydrochloric acid and diluted to 10 ml. with a sodium chloride–ammonium chloride buffer solution. The spectrum of 0.1 ml. of this solution is excited in a 15-ampere, direct current graphite arc. The analysis is made upon the basis of a microphotometric comparison of the blackenings of the lines of the test elements in the specimen with those in standard solutions. The analytical error is ordinarily less than ±10 per cent of the amount present.

TABLE VIII. ANALYSIS OF GEOCHEMICAL MATERIALS

Test Element	Lowest Concentration Determined	Sensitivity (Element on Electrode)
	%	*Mg.*
Li	0.000047	1.4×10^{-6}
Be	0.00036	1.1×10^{-5}
B	0.00016	4.7×10^{-6}
La	0.00085	2.6×10^{-5}
Co	0.0037	1.1×10^{-4}
Ni	0.0005	1.5×10^{-5}

GEOCHEMICAL SAMPLES. Investigation of the abundance and geochemical distribution of the chemical elements required the development and use of sensitive physical methods of quantitative analysis of rocks, minerals, glasses, slags, ashes, clays, and soils which would determine most elements down to concentrations of 0.001 per cent or less. Spectrographic methods that utilize the excitation of spectra in the cathode layer of the direct current carbon arc have successfully fulfilled these requirements (*16*). Representative examples of such analyses are given in Table VIII.

In analysis for lithium in mineralogical specimens a few milligrams of the sample are ground and mixed with strontium oxide, the internal standard, and approximately 3 mg. of the mixture are packed into a cavity in the negative electrode of a 10-ampere carbon arc. The photometric method is similar to that used for the analysis of caustic liquors. A single spectrum gives a possible error of ±25 per cent, while the average of four spectra limits the error to less than ±5 per cent. One operator can make 64 analyses per day.

This technique yields a rapid, precise, highly sensitive, quantitative analysis of a few milligrams of chemically untreated mineralogical sample.

Acknowledgment

The writer is indebted to T. M. Hess for many helpful discussions during the preparation of this manuscript.

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Fluorescent Analysis of Inorganic Materials

C. E. WHITE, University of Maryland, College Park, Md.

FLUORESCENT analysis has been given added impetus in recent years by the contributions of a great many workers. The reviews prepared by Radley and Grant (*19*) in England, and Haitinger (*13*) and Danckwortt (*5*) on the continent have assisted greatly in focusing attention upon this subject. The recent interest of textbook editors in fluorescent analysis is attested to by the fact that Feigl (*8*) in the 1938 edition of his text on Spot Test Reactions has included a section giving a general discussion of the technique of fluorescent analysis; and under the specific test for the elements he designates fluorescent methods in at least a half dozen cases. In some instances the tests are entirely new, while in others the sensitivity is greatly increased by examination in ultraviolet light.

It is admitted that as an inorganic laboratory tool fluorescence is still in its early stage of development. The progress has been handicapped by inconvenient light sources and apparently limited applications.

It is the object of this paper to outline the progress in this field, with the hope that it may stimulate an interest which will lead to a broader use of this interesting phenomenon in inorganic work. The general applications of fluorescent analysis in organic chemistry, in criminological work, in biology, medicine, and pharmacy, and in other branches of science will not be included. However, it is sometimes difficult to limit a field of this sort—for example, the organic chemist may use an inorganic substance in identifying his compounds; or as in fluorescent chromatographic analysis, an inorganic absorbent is used to separate the organic materials.

The term "fluorescence" is a broad one, embracing secondary rays of many different wave lengths, and is used chiefly to designate the visible light emitted when a substance is brought under the influence of an invisible exciting source. Many of the secondary rays produced may be too short or too long for direct identification by the eye, and a wave motion between 4,000 and 8,000 Å. may cause the emission of another visible

ray; but present analytical applications of these extremes are so limited that they will not be considered.

Sources of the Exciting Ray

Fluorescence may be excited in many different ways: by cathode rays, radium rays, x-rays, etc. A recent article (*9*) indicates that cathode rays were used in studying the fluorescence of eighty specimens of calcite. It is, however, the near ultraviolet that is the most generally applicable, and the discussion here will be confined to the methods of producing the rays essentially between 3,000 and 4,000 Å. In any case the excitation source should always be carefully described, as some of the contradictions appearing in the literature may be due to the use of varying intensities of ultraviolet rays. In general, the strongest source available should be used so that no obscure phenomenon may be overlooked.

The spark or arc discharge between metallic electrodes such as iron, nickel, cobalt, aluminum, tungsten, magnesium, and cadmium, and the impregnated electrodes where the core contains aluminum, tungsten, etc., provides an intense source and one rich in the shorter wave lengths. The arcs have been developed to a point where they are slow-burning and may be used for some time without attention. The iron arc is often recommended where observations are to be made under a microscope, since the ultraviolet must travel by way of a lens and prism or mirror before it is used, and an original intense source is necessary. Of the metals mentioned iron, tungsten, and molybdenum give the greatest number of lines between 2,000 and 4,000 Å. Practically all the metallic and impregnated carbon electrodes give resulting spectra with greater number of lines and with greater intensity than the mercury arc.

The disadvantages of the electrode arc lamps are well known, but with proper equipment such as that developed by Reichert, the electrodes can be handled in a satisfactory manner.

For this so-called Haitinger-Reichert lamp, Haitinger has developed an iron electrode, consisting of an iron tube packed with an iron and carbon core, which burns smoothly and does not form the iron oxide film as does the ordinary rod electrode. The current consumption of this lamp is only 4 amperes on direct current or 8 amperes on alternating current, which means that the metal electrodes will burn away very slowly. The lamp will burn for hours at a time without adjustment of the electrodes.

Another type of iron arc lamp, a vacuum arc, has been advertised recently by Kipp and Zonnen. This was developed at the suggestion of Professor Zeeman of Amsterdam and is easily evacuated to a pressure of 4 cm. of mercury with an ordinary filter pump. The arc burns slowly in a constant way.

Next to the arc lamps the quartz mercury vapor lamp is most favored.

The analytical model manufactured by the Hanovia Company is widely used. This lamp is designed with a cylindrical hood and special reflectors which greatly increase the efficiency of the quartz mercury arc source. The front of the lamp is fitted with an easily removable filter which removes most of the visible radiations. The intensity of this lamp is reported to decrease slowly during the first 400 hours of operation and then remain practically constant for several thousand hours of use. The short wave lengths emerging through the quartz cause a considerable quantity of ozone to be formed, and since this gas is poisonous even in small quantities some means should be provided for its removal. An ordinary ventilating fan is satisfactory for this purpose.

There are many models of mercury vapor lamps on the market and most of these will give some degree of satisfaction. Another type of mercury vapor lamp is known as the high-pressure mercury arc lamp, such as the H4 of the G. E. Vapor Lamp Company. One important asset of these lamps is their simple operation and low original cost. With a small ballast transformer they operate on any 110-volt line. Their greatest intensity in the ultraviolet is, in general, around 3,660 Å. which is ample for most routine laboratory work. The outside envelope may be entirely removed or a hole bored in it to obtain radiations below 3,500 Å. If the lamp is enclosed so that the visible rays are removed by a filter it must be cooled by air circulation so as to approximate conditions in an open room. Commercial units of this sort are available. Some of the mercury vapor lamps are made with dark glass so that they may be applied directly in fluorescent work. These are designed especially for demonstration purposes and are not recommended for general laboratory practice.

Another source of the near ultraviolet which will produce fluorescence is the well-known argon bulb. The intensity of this is so low that its application is limited. For example, some tests which are good to 1 part in 10,000,000 under the quartz mercury vapor lamp will detect only 1 part in 100,000 with the argon bulbs. A battery of these bulbs has been applied in the quantitative determination of riboflavin (*24*). Here riboflavin is compared to a fluorescein standard and the authors claim excellent results.

Filters

In considering a source of ultraviolet rays the filter used becomes an important factor and its type and thickness should always be designated. By reference to any manufacturer's catalog of glass filters, such as Corning or Jena, one may determine the type desired for a particular job. Where intensity is a factor the thickness of the glass is also significant—for example, an 8-mm. nickel oxide glass decreases the intensity of the iron arc too greatly for some microscopic examinations, and 3 mm. permits the passage of too much visible light. A 5-mm. filter serves as a compromise. In addition to glass filters, gelatin, Cellophane, and colored solutions such as those of copper and nickel salts, are employed with much satisfaction. A list of solutions to isolate the important lines between 2,480 and 5,790 Å. has been published (*2*, *7*, *22*).

The intensity of the ultraviolet light used may be determined by chemical reactions, spectrographic methods, or photronic cells. The Westinghouse Company has designed a photoelectric apparatus for this particular purpose. It is believed, however, that if the type of lamp, the filter, and distance of operation are specified, it is not necessary to report the actual intensity of the ultraviolet light.

Condition of Sample

The sample to be observed may be in the solid, liquid, or gaseous condition. If the substance is a solid, the size of the particles becomes important. Too large or too small a particle may not fluoresce at all, or may have a different appearance from one of intermediate size. Borax, phosphate, or fluoride beads serve well for examining many inorganic substances. In the liquid examinations the solvent should be nonfluorescent if possible. A solvent fluorescing in the green may completely mask a substance giving a red fluorescence. The concentration, temperature, and acidity of the solution all have some effect upon the luminescence. In the case of the concentration the result is fairly obvious, the nature of the color does not change, but the intensity fades with increasing dilution. In a recent article on the fluorescence of the rare earths (*21*), the authors indicate that a wide variety of temperatures was used in their study. The acidity of the solution is of some moment. Some materials not fluorescing at all in a neutral solution may do so in an acid or alkaline medium. In the morin test for aluminum, scandium, gallium, and indium Beck (*1*) has shown that the fluorescence is strongest in a little mineral acid. Sodium acetate and sodium fluoride weaken the fluorescence in this case. Materials responsive to oxidizing and reducing agents are, of course, affected if these are present—for example, it is only the lower valence of mercury, copper, and tellurium that fluoresces.

Quartz containers are the best for transmitting the ultraviolet. However, if an ordinary filter is used on the lamp source, one may just as well use glass containers, since the lower limit of transmission is about 3,000 Å. in both cases. The fluorescence of the container should always be tested before attempting to use it in analysis. Ordinary white spot plates will fluoresce in the purple, and black ones in the green. Glass spot plates can be used satisfactorily. The fluorescence of a material may be observed sometimes much better in a test tube than on a spot plate. This is true in the case of the Blue Black R test for aluminum. The test is visible only with transmitted light. Spot test paper, either black or white, is satisfactory in many cases. Here again the fluorescent property of the paper and also the reagent must be tested. Some reagents, especially the dyes, will not fluoresce in water solution but show a brilliant fluorescence when placed on spot test paper. Minute quantities of solutions may be tested by placing a drop between quartz microscope slides and observing this in both reflected and transmitted light either with or without the microscope.

Reichert (*20*) has designed an excellent apparatus for microscopic observations. In this arrangement the light passes through a collecting lens system, a glass filter, and a copper sulfate solution filter. The latter removes all of the red rays and this prevents undue heating of the object. Filters are used on the eyepieces to remove the wave motions below 4,000 Å. which are likely to injure the eye. The microscope may be provided with a spectroscopic or spectrographic attachment, so that the fluorescent light may be analyzed. If the solution is allowed to crystallize, the fluorescence of the crystal is often much more intense than that of the solution. Attachments for fluorescent work may be placed on almost any microscope. Both incident and transmitted ultraviolet arrangements are used.

For examination in a macro way the containers may be placed directly under or in front of the lamp. The arrangement used by Haitinger and Reich (*15*) is satisfactory. Here the ultraviolet light enters the top of the tube and the depth to which it penetrates depends somewhat on the concentration.

The fluorescent color should always be described in terms of Angstrom units. It is rather indefinite to say that a material gives a green fluorescence, since there is such a wide variety of greens and it is difficult to describe a particular shade. The use of a spectroscope, spectrograph, colorimeter, photometer, or Lovibond tintometer will more accurately convey the idea of the region of the spectra involved.

Quantitative Methods

Quantitative methods using fluorescence have been applied successfully in organic analysis, but little has been done with inorganic materials. We have already mentioned the simple method used in the determination of riboflavin. Cohen (*4*) in Holland has applied the selenium photronic cell to the measurement of fluorescence of lactoflavins, and this method might be applied to inorganic solutions. Results were satisfactory to 2×10^{-5} gram per ml. and were accurate within 3 per cent. The curve which Cohen obtains from his data is typical for fluorescent solutions. At the lower concentrations there is a straight-line relationship between intensity and concentration, but at higher concentrations the solution reaches its saturation point as far as fluorescence is concerned. As far back as 1925 Lutz (*16*) reported a quantitative fluorescent micromethod for zinc, where he used urobilin as the reagent and compared the intensity with Nessler tubes. The method will detect 0.01 to 0.5 mg. of zinc in 50 ml. within a 10 per cent error.

The Pulfrich gradation photometer gives a rapid and satisfactory method of determining concentration from the intensity of the fluorescence. In the recommended apparatus both liquid and solid filters are used, and a special glass is inserted to remove any ultraviolet before it reaches the eye of the operator. Cells of any size may be used and this may be classed as a micromethod. The high light-transmitting power of the Pulfrich is especially advantageous for measuring the feeble intensities of fluorescent light. The Pulfrich is also adaptable to the measurement of the fluorescence of solids. In this case a piece of uranium glass or other solid may be used as a standard, and by interposing correct filters the intensity of any fluorescing color may be measured.

Matheson and Noyes (*17*) use a photoelectric cell and a DuBridge circuit with an F. P. 54 tube for measuring the fluorescence of acetone. Byler (*3*) uses the MacBeth illuminometer manufactured by the Leeds & Northrup Company to measure the intensity of the fluorescence of calcium phosphates. With this instrument he measures intensities as low as about 0.6 per cent of grade A zinc sulfide and differences as low as 0.08 per cent.

Spectrographic and photographic methods may be used in quantitative measurements, but these seem to be more or less troublesome.

Confined spot tests, as developed by Yagoda (*26*) and capillary adsorption on filter paper should find some semiquantitative application for inorganic fluorescent analysis.

Applications

The inorganic materials that fluoresce are rather well classified by Radley and Grant (*19*). Fluorescent methods are not generally applied in mineral identifications. Of the many known minerals only about forty are fluorescent and the mineralogist knows these so well that special methods are not necessary. However, the detection of traces of elements in minerals may sometimes depend on fluorescent methods. Haberlandt and others (*12*) claim that the fluorescence of fluorite is due to divalent europium, because europium chloride gives the same bands in both the red and blue as does fluorite. The blue band is sensitive to 10^{-6} gamma. A fluorescent study of sapphires and rubies (*23*) indicates that they both contain the same element, probably chromium.

Uranium is most easily detected in traces by the strong fluorescence of solid uranyl salts; 0.001 gamma in a concentration of one in a million is evident. This finds application also in the zinc uranyl acetate test for sodium, which is made much more sensitive if the material is examined in ultraviolet light. In ordinary light the limit of the test is 12.5 gamma at a concentration of 1 in 4,000, in ultraviolet 2.5 gamma at 1 in 20,000. The fluorescence of uranyl salts is decreased by the presence of strong oxidizing or reducing ions. The inhibition of fluorescence by added agents seems unpredictable. Addition of 0.5 part per million of nickel to zinc sulfide prevents its fluorescence. Ozone has been determined by its decreasing the brilliance of fluorescein. Activators operate in an opposite manner. Copper and manganese seem to increase the fluorescence of zinc sulfide. It is believed that traces of activators or inhibitors can be detected by their effect upon fluorescent material sufficiently for a qualitative identification.

The effect of pH changes on fluorescence is so striking that this may be used to determine the end point of a titration. Déribéré (*6*) gives a table of thirty-seven organic substances with their pH limits and the effect of oxidizing and reducing agents. There are many interesting applications of indicators of this type. Gotô (*10*) employs alpha-naphtholflavone for iodometry. The blue fluorescence of this substance disappears in the presence of free iodine or bromine and reappears when these halogens are removed. This can be used in the titration of iodine by sodium thiosulfate and in the titration of arsenious acid by potassium bromate. Ohac (*18*) titrates Al^{+++} with sodium fluoride, using morin as a fluorescent indicator. In these titrations a small vial of quinine sulfate may be floated in the buret to read the meniscus.

In most inorganic tests using organic materials the pH must be somewhat controlled. The molybdenum test using tincture of cochineal which is sensitive to 0.02 gamma of molybdenum oxide must be carried out at a pH between 5.7 and 6.2.

It is hoped that fluorescence will add to the specific test for the elements. Such a case is illustrated in the Pontachrome Blue Black R (*25*) test for aluminum, which is sensitive to 0.2 gamma of aluminum-ion concentration and to a dilution of one part in ten million. It serves to distinguish aluminum from all other elements investigated, and is the first direct chemical test to differentiate it from beryllium. The morin fluorescent test for aluminum is more sensitive and will detect 0.05 gamma at 1 part in 10,000,000 but is given also by beryllium, indium, gallium, and the rare earths.

In addition to the examples given, fluorescent tests, all of microapplication, have been worked out for beryllium, zinc, arsenic, tin, bismuth, manganese, cadmium, columbium, H_3BO_3, and H_2SO_3. A detailed description of these is given by Haitinger (*14*). Gotô (*11*) lists fluorescent methods for about twenty-one elements. This indicates fair progress in the development of fluorescent determinations. Fluorescence is in position at present to claim precedent over standard procedures in only a few cases. Indications are, however, that many more applications will be forthcoming and fluorescence will take its place in general inorganic analysis.

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Isolation and Determination of Traces of Metals

The Dithizone System

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COMPLEX organic compounds have become increasingly popular for qualitative and quantitative determination of metals. Such compounds, sometimes highly colored and often soluble in organic solvents, under definite conditions form complexes with certain metals that are likewise often highly colored and soluble in solvents immiscible with water. It is the favorable extraction coefficient, the intense color, or both, that has greatly interested analysts. Many convenient analytical separations have been based on such extractions even when the complexes produced were colorless, and when they possessed a high degree of color, colorimetric methods, often of surprising accuracy, were developed soon after the discovery of the complex. These newer chemical methods have been used to determine smaller and smaller quantities of metals, until what was considered a microquantity a few years ago is now lightly spoken of as almost of macro proportions. It was even necessary to use new units of measurement. One speaks now of micrograms or gammas to divide the milligram, the microchemical unit of former days.

Diphenylthiocarbazone (phenylazothionoformic acid phenylhydrazide), usually abbreviated to "dithizone," is a highly colored organic compound that produces brilliant yellow, red, or violet colored complexes with a dozen or more metals. It is exceedingly useful in extracting a whole group and, under the proper conditions, separating subgroups and determining the individuals thereof. Dithizone is therefore not a specific reagent, but under specific conditions it has become an excellent example of recent trends in chemical analysis and a new tool that has enabled chemists interested in the isolation and determination of traces of metals to extract and determine rapidly near-spectroscopic as well as milligram quantities of certain metals with an accuracy greater than that found in spectroscopic determinations, and without highly expensive apparatus or extensive experience. Herein lies its great value. This is not an implication that dithizone methods will ever supplant the spectroscope. The most useful range of dithizone methods is from 1 to 200 micrograms, and the errors vary from less than 1 per cent to 5 per cent, depending somewhat on the amounts to be determined. Therefore it may be said that dithizone methods begin about where spectroscopic methods usually leave off, and the two should be able to exist side by side. The dithizone system of analysis, however, does give the chemist in the ordinarily equipped chemical laboratory an excellent chance to compete in a complicated and highly technical microchemical problem at the added expense only of preparing slightly larger samples and taking extra precautions in the purification of reagents and prevention of contamination. Perhaps the same might be said about other purely chemical analytical systems of like sensitivity, but the dithizone system with its brilliant display of colors has certainly captured the imagination of a great many analysts, and has become the principal contender in this special field.

Hellmut Fischer, a metallurgist, is the father of the dithizone system of analysis. He and his co-workers have published numerous papers on the analytical properties of dithizone and its use in microanalysis, and a detailed review of dithizone literature up to 1938 from Fischer's pen may be found in *Angewandte Chemie* (*9*). In the United States the biochemists, pharmacologists, toxicologists, and food chemists have exhibited the greatest interest in dithizone, with the determination of lead as the focal point. This paper was written in an attempt to stimulate interest among other American chemists, especially the physical chemists, in this analytical field, and to promote the determinations of other "dithizone metals" besides lead. Attention will therefore be directed towards principles, and certain gaps in our present information will be emphasized with the hope that greater progress will be made. Perhaps a few speculations concerning future developments may also be of interest to this audience.

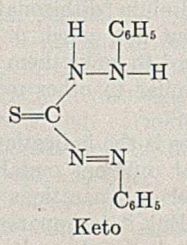
Analytical methods are divided into three parts—preparation of sample, isolation of the element sought, and its final determination. This paper is, therefore, divided in a corresponding manner.

Sample Preparation

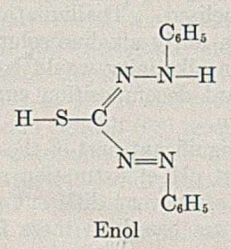
A discussion of sample preparation, especially in the case of biological material, is important, but for the purpose of this paper may be brief. Inorganic substances are usually dissolved in acids without much difficulty. Organic matter is destroyed by either wet or dry ashing where this is necessary. The dithizone system of analysis, being based on an extraction process, does not always require destruction of organic matter—for example, over 100,000 lead determinations have been made in the United States in the last year, on sprayed apples and maple sirup, wholly or partly by dithizone methods, without destruction of organic matter. In fact, a vigorous partial oxidation of organic matter with nitric acid, filtration, and aliquoting is often all that is necessary for sample preparation prior to a lead assay. The nitric acid brings insoluble lead compounds into solution, and breaks up or destroys colloids that cause emulsion formation or other difficulties in subsequent extractions of the metals with dithizone in organic solvents. Of course such treatment is not practical in all cases, and where it is not, some system of wet or dry ashing must be employed.

Separations

Assuming that the sample has been properly prepared, the next step is the isolation of the metal desired, from aqueous solutions often containing interfering metals, as well as acids, salts, and sometimes organic matter. Since dithizone lacks specificity, separations are highly important and require careful consideration. Certain properties of dithizone and its complexes that influence separations are therefore discussed here.

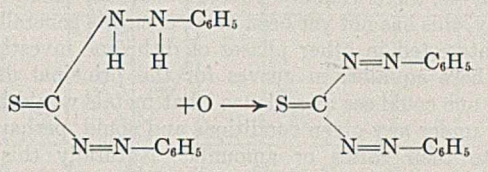


Keto



Enol

Diphenylthiocarbazone exists, according to Fischer (*8*), in keto or enol form, the former being the more important analytically. It is very insoluble in water but soluble in ammonia and in many organic solvents, carbon tetrachloride or chloroform being the most convenient and practical ones. Fischer (*9*) believes that it is oxidized by mild oxidizing agents to the yellow, non-complex-forming, chloroform-soluble but water- and alkali-insoluble, diphenylthiocarbadiazone which may again be reduced to dithizone by reducing agents like hydroxylamine hydrochloride, or sulfites. American experience indicates that the two reducing agents mentioned are useful in preventing oxidation of dithizone. Stronger oxidation may attack the sulfur or break the compound at other places, with irreparable damage. A solution of pure dithizone in chloroform or carbon tetrachloride is stable if protected from direct sunlight and kept cool. Clifford (*3*) finds that overlaying a stock solution of dithizone in carbon tetrachloride with a 0.1 molar solution of sulfur dioxide preserves it unchanged for months if stored in the dark at ice-box temperatures.



Dithizone in solution has a tremendous tinctorial power and appears red or green, according to the concentration or the depth of the column through which it is viewed. It forms yellow, orange, red, or violet complexes with a dozen or more metals, almost all readily soluble in chloroform but less so in carbon tetrachloride. The "dithizonates" are simultaneously formed and extracted in most instances (platinum, palladium, and gold form colored flocks in carbon tetrachloride) by shaking aqueous solutions of the metals, at the proper hydrogen-ion concentration, with chloroform or carbon tetrachloride solutions of dithizone. The green color of excess dithizone in chloroform modifies the color of the extracted dithizonates with the production of beautiful so-called mixed colors ranging from green through blue, purple, and crimson to red in the case of the red dithizonates, according to the relative quantities of metal and excess dithizone. Mixed colors are not produced from alkaline aqueous solutions with carbon tetrachloride solutions of dithizone because dithizone is less soluble in that solvent, and the excess will largely dissolve in the aqueous phase.

Because both dithizone and the dithizonates are very soluble in chloroform and very insoluble in water, there is a most favorable partition coefficient if the hydrogen-ion concentration has been properly adjusted. Since the extracting solvent is heavier than water, repeated extraction can be made in separatory funnels without transfer of the solute. Minute as well as comparatively large microquantities of metals can therefore be extracted from even large volumes of aqueous solution by a process of "extractive enrichment," to use Fischer's expression (*7*). It is a matter of controlling the comparative volumes of the two phases and especially the concentration of the dithizone in the chloroform phase and the pH of the aqueous phase.

The various metals of the dithizone group react at different optimum hydrogen-ion concentrations of the aqueous phase. Therefore the pH governs the order in which the metals are extracted. Generally the more "noble" a metal is, the lower is its pH for optimal extraction. The metals, gold, platinum, palladium, silver, mercury, stannous tin, copper, bismuth, zinc, cobalt, nickel, lead, thallium, and cadmium react with dithizone in immiscible solvents more or less consecutively as the reaction of the aqueous solution is progressively changed from strong acid through weak acid, neutral, ammoniacal, and alkaline conditions up to 5 per cent of sodium hydroxide, but there are numerous coextractions.

Extractions of metals may be made at unfavorable pH with decreased efficiency, which may be in part overcome by paying special attention to the restriction of the volume of aqueous solution, increasing the volume and especially the concentration of the dithizone solution, and by vigorous agitation to bring the reactants into equilibrium. The separation of the different dithizone metals at any given pH is also modified by the relative quantities of the metals. The extraction of lead at pH 4, for example, is unfavorable, while that of mercury and copper is favorable. The mercury will tend to extract first and then will come the copper; but if the quantity of lead is large as compared with the other two, it may contaminate them to a certain extent.

If the effects of concentration of metals and of dithizone and hydrogen-ion concentration on the percentage of metal or metals extracted could be expressed in the form of equilibrium curves, there would be immediate use for them. Unfortunately, this has not yet been done, chemists generally being more interested in other phases of dithizone investigations. Were there equilibrium curves for the principal dithizone metals, one could see at a glance what metals would be coextracted under any given conditions and could, perhaps, even calculate their ratios or amounts. Naturally this would simplify separation problems. The Willoughby (*28*) separation of bismuth and lead at pH 2 is effective and illustrates what can be done by empirical methods. Were exact equilibrium curves available, they should show clearly the reason for this effectiveness, and the maximum departure from pH 2.0 permissible, to improve the ease of the extraction of bismuth without consequent loss of lead. Control of hydrogen-ion concentration is therefore the sieve that makes the first approximate separation of the dithizone metals.

The various dithizonates, when once formed and dissolved in the solvents under optimum conditions, vary in their stability toward acids. Lead, with an optimum extraction at pH 9.5, can be easily retransferred to the aqueous phase by shaking the chloroform solution of lead dithizonate with dilute acid. The alternate solution of lead in chloroform and acid phases can be repeated as often as wanted, and such alternate extraction may be utilized in the separation of lead from interferences of either metallic or nonmetallic nature. Zinc may be retransferred to the aqueous phase in a like manner, but it usually requires a little stronger acid or more shaking. Certain other dithizonates are comparatively stable towards dilute acids when once dissolved in chloroform and re-enter the acid phase with varying degrees of reluctance. Silver, mercury, and cobalt require rather strong acid to force them into the aqueous phase, while copper and nickel are more amenable to its action. A further study of the equilibria involved herein should pay dividends.

The metals also differ from one another in the stability of their dithizonates towards alkali. Lead dithizonate in chloroform solution becomes unstable towards aqueous alkali solutions at pH 11 or above and the lead begins to return to the aqueous phase where it may partly precipitate as the hydroxide. Bismuth and tin dithizonates will decompose, and the metals will return to the aqueous phase at a pH of 9 to 10. The critical pH governing the stability of zinc dithizonate is probably near 10.0, that of thallium near 11.0, and cadmium 12.0 or above. Bismuth and tin which are extracted with lead by dithizone have been separated from that metal by washing the dithizonates with diluted ammoniacal solutions at a probable pH value of about 10. The nature of the solvent also has an influence on the action of aqueous alkalies on dithizone and the dithizonates. Lead dithizonate in chloroform is more stable towards alkaline solutions than in carbon tetrachloride, the reversion point being approximately at pH 11.0 as contrasted to about pH 10.0 (*20*). Hellmut Fischer and associates, and other writers, have based determinations of metals on washing the excess dithizone from carbon tetrachloride or chloroform solutions of dithizonates with weak ammonia solutions of 0.01 to 0.04 normality or mixtures of ammonia, cyanides, and sometimes ammonium chloride. It is admitted that the use of washing solutions that are too alkaline may result in metal losses due to the decomposition of some of the dithizonates, but the equilibria that govern the reversion of dithizonates by aqueous alkaline or acid solutions have been studied just as inadequately and empirically as those that produced them. Progress, therefore, seems to demand that the underlying principles be examined carefully, preferably by physico-chemical methods.

Before planning extensive equilibrium experiments the present state of our knowledge should be appraised. The solubility of the dithizonates in organic solvents depends upon a number of simultaneous equilibria, depending on concentration of metals and dithizone, variation in hydrogen-ion concentration of and presence of complex-forming salts in the aqueous phase, and partition coefficients of dithizone and dithizonates between solvent and aqueous phases. Such a complex system of simultaneous equilibria cannot be expressed in simple equations or curves. Nevertheless, the necessary basic ideas may be formed and much useful information may be gained by reducing the problem to the simplest terms. A beginning has been made with lead (*6*) by determining the percentage of lead extracted from a definite volume of aqueous solution of definite metal salt concentration by a definite excess of dithizone in chloroform over a useful hydrogen-ion concentration range. Such a system can be expressed by two coordinate curves. Increase or decrease of the metal or dithizone concentration would produce a family of curves by shifts towards the left or right, respectively.

Carbon tetrachloride as solvent would probably shift the curves about one pH unit to the left. Figure 1 shows a rough approximation of our present fragmentary knowledge. No pretense of accuracy is made in the drawing of these curves, since the basis for most of them is their analogy to lead and some bits of information picked here and there from the literature. These double sigmoid curves explain in a fashion many of the empirical facts found up to the present time in the development of dithizone methods of metal analysis. The writer hesitates to express an opinion on the ultimate shape of these curves when all the factors entering into the equilibrium are accounted for. Anyway, a consideration of the present curves gives useful information and furnishes a basis for an estimate of what the future may have in store. The curves of the metals shown in Figure 1 are based on the keto combination with dithizone. Silver and mercury are extracted from fairly acid solutions and therefore their curves are not expected to pass through the origin. In alkaline solutions beyond approximately pH 11.0, silver, mercury, and copper tend to produce the enol modifications, but since the conditions for this transition with respect to pH are not well known, only the acid extraction is symbolized. Thallium and cadmium dithizonates are stable in fairly alkaline solutions, but how much of an increase in alkalinity would be required to make them exhibit downward decomposition curves similar to other metals is unknown.

The significant part of these curves is the indication of an optimum pH of extraction and that different metals react best at often very different pH levels. Separations by pH control are based on these facts. But it is apparent that clean-cut separations of metals can be made only if the upper and lower parts of their curves (the future successors to the curves in Figure 1 are meant) do not overlap at the pH chosen for the extraction. When there is an overlapping indicating the probability of a small coextraction of an inter-

fering metal, such interference may possibly be reduced to negligible proportions by a number of alternate transfers between aqueous and immiscible solvents. Such a procedure resembles the double precipitation of macroquantitative analysis. The other alternative would be the extraction of the desired metal at a pH not optimum but where there is no overlapping. In any event, it does not need much imagination to realize that if we could replace this crude sketch with equilibrium curves based on accurate data, the problem of separations in either acid or alkaline solution would be much simplified. The attention of physical chemists is invited towards this interesting problem.

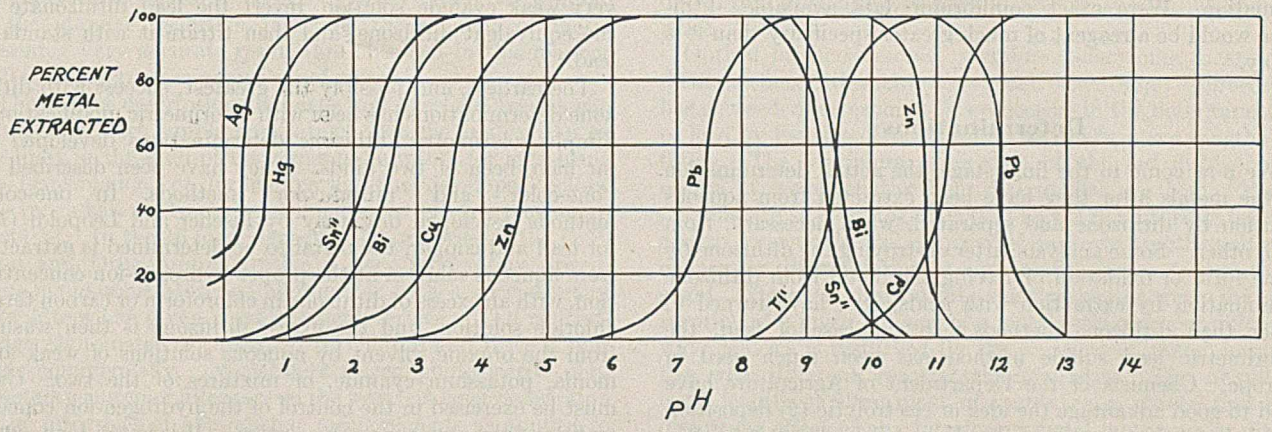


FIGURE 1. PROVISIONAL EQUILIBRIUM CURVES OF SOME METAL DITHIZONATES IN CHLOROFORM

The dithizone system has another string to its bow in the matter of extractions and separations. The second string is the relative stability of other complexes towards the dithizone complex. Citrates or tartrates are used to prevent the precipitation of hydroxides or phosphates when dithizone metals are extracted from alkaline solutions, but do not hinder the dithizone extraction. Fischer and Leopoldi (*10*) originated the use of cyanides as a discriminative complex former, or masking agent. In weakly ammoniacal solutions the double cyanides of most of the dithizone group of metals are stronger complexes than the dithizone complexes. The exceptions are lead, bismuth, stannous tin, and thallium. Therefore, the last three dithizonates are coextracted with lead dithizonate from cyanide solutions and interfere in the determination of lead. Fischer and his associates (*11*) also determined that potassium iodide and sodium thiosulfate were excellent competitive complex formers for many of the dithizone metals in acid, but lost this power in alkaline solution. Although the optimum pH for the dithizone extraction of zinc is about 7.0, Fischer *et al.*, nevertheless, were able to develop an excellent method for the determination of zinc by the so-called *Tarnung* (concealment, masking, camouflage) of interfering metals with thiosulfate and potassium cyanide at a pH of 4 to 4.5. Cadmium seemed to be the only metal that gave them trouble, if present in more than 100-microgram quantities. Workers in the United States used thiosulfates and potassium iodide in dithizone methods at about the same time that Fischer did. Winkler (*29*) and Sandell (*23*) recommend these reagents in the dithizone determinations of mercury, copper, lead, and zinc. Thiosulfates and iodides in acid solutions will also revert some acid-stable dithizonates and retransfer the metals, now combined in the stronger thiosulfate or iodide complex, from the organic solvent to the aqueous phase. Winkler finds that the mercury-thiosulfate complex can then be readily broken by oxidizing the thiosulfate to the non-complex-forming sulfate, which frees the mercury for a later determinative dithizone extraction.

Recently, organic competitive complexes have found a place in the complex fixation of dithizone interferences. Ritchie, as associate referee for the Association of Official Agricultural Chemists, and his associates (*22*) recommend diethyldithiocarbamate, the copper reagent, for interference fixation in the determination of zinc. They first extract all possible interfering metals as carbamates and dithizonates with carbon tetrachloride from aqueous solutions too acid for the extraction of zinc. They then fix remaining interfering metals other than zinc with the carbamate in weak alkaline solution. It seems that under these conditions dithizone becomes almost a specific for zinc, cobalt and nickel being the only metals that will require further study.

Another use of organic complexes in lead determinations has been made in the laboratory of the Food and Drug Administration (*3*). Iron (ferric iron does not react with dithizone but the ferricyanide formed with potassium cyanide oxidizes dithizone), tin, and about 80 per cent of the bismuth in 100 cc. of acid solution are precipitated by cupferron and then extracted with ether or chloroform, which leaves the soluble cupferrides, including lead, in the aqueous phase. After freeing the lead from the cupferride complex by bromine treatment, it may be extracted later on from ammoniacal solution, separated from residual bismuth, and then determined. This appears to be a very clever short cut to separate almost all the interfering metals from lead in one operation before dithizone is even applied.

There are other complexes besides those mentioned that are, or may become, useful in the dithizone system. A desirable competitive complex in any dithizone determination should have a strong fixing power for as many interfering metals as possible; and little or none for a limited number of other metals including the one that is being determined, diminishing thereby the number that have to be separated by other means. If the pH range for the competitive complex is wide enough to allow separations of the now lesser number of dithizone extractable metals by hydrogen-ion concentration control, an almost specific dithizone method for a given metal may be the result. Another consideration that may govern choice of a competitive complex is the ease with which it may be removed, destroyed, or inactivated when it has performed its mission, and its continued presence becomes embarrassing later on in the determination. A greater number of complexes to select from should promote progress in dithizone methods.

The principle of competitive complexes may find application with other organic reagents. Homologs of dithizone might also alter the picture. Future developments may take unforeseen directions, but the important points that may be

emphasized now, in the separation and isolation division of the dithizone system of analysis, are that the dithizone group of metals can be extracted from aqueous solution and separated from each other by a careful control of hydrogen-ion concentration, by competitive complex formation, or by a combination of these principles. It is certain that physical-chemical investigations of the various equilibria involved could help this analytical problem greatly by enlarging and placing on a firm theoretical basis our present empirical information. Were exact equilibrium data available, dithizone would be a reagent of much greater specificity than it is today.

Determinations

We now come to the final stage, the actual determination of the metals after they have been extracted from aqueous solution by dithizone and separated, when necessary, from each other. Some analysts, after destroying the dithizone by some form of oxidation or freeing the metal from dithizone combination by extraction with acids, etc., have turned to other than dithizone methods. In the case of lead, the colorimetric lead sulfide method has been much used in Europe. Chemists of the Department of Agriculture have used to good advantage the idea of electrolytic (*2*) deposition and iodometric titration of the deposited lead peroxide after a preliminary dithizone extraction. Perlman and Mensching (*21*) in their work on the determination of zinc in maple products and Sylvester and Hughes (*24*) in England have used dithizone to extract zinc usually contaminated with other metals from aqueous solution, and then determined the zinc iodometrically with ferricyanide and potassium iodide according to the Lang (*17*) method, which is said to be specific for zinc. One cubic centimeter of 0.001 *N* thiosulfate is equivalent to 100 micrograms of zinc. The method is, therefore, rather insensitive in the lower range of zinc (1 to 100 micrograms), and this lack of sensitivity must be compensated by larger samples.

Dithizone is readily oxidized, and Hibbard (*14*) has utilized this property in the determination of zinc after extraction as the dithizonate. He oxidizes the separated zinc dithizonate with an excess of standard bromine in carbon tetrachloride solution and then back-titrates the excess bromine with potassium iodide and thiosulfate. Hibbard believes his results for 10 to 30 micrograms of zinc, in the absence of interfering substances, are accurate to ±10 per cent. The determination of other dithizonates can be made in this manner. Hibbard suggests the determination of copper, lead, cobalt, and cadmium. Limiting factors are the purity of the separated dithizonate solutions and the sensitivity of the final iodine-starch titration.

The current analytical methods, based entirely on the use of dithizone, may be classified as extractive titrimetric, or colorimetric (several varieties). Extractive titration methods involve the extraction of metals from aqueous solution at definite pH, and in the presence of fixation complexes when necessary, with successive increments of standardized dithizone solution. The titrations are made in separatory funnels with sufficient shaking between additions to establish equilibrium between the metal and dithizone, and the solvent layer containing the dithizonate is drawn off from time to time, until the green dithizone is no longer changed in color. The intense green dithizone, therefore, furnishes its own end point. The dithizone solution is standardized against known amounts of metal in the same manner.

This principle has been applied to silver and other metals with most excellent results by Fischer *et al.* (*13*). They sometimes titrate silver, not with solutions of dithizone, but with solutions of copper dithizonate and declare that interference from other metals is less. They also determine other metals indirectly by a silver titration. In the United States the principle of direct extractive titration has been applied by Winkler (*29*) to mercury and by Wilkins, Willoughby, *et al.* (*27*) to lead. The accuracy of the extractive titration methods is of the order of about 1 microgram.

A variation of the above direct titration with respect to lead has been published by Horwitt and Cowgill (*15*). They extract with excess dithizone, remove the excess dithizone with very weak cyanide solution, revert the lead dithizonate to the equivalent dithizone, and then titrate it with standard lead.

The earliest, and possibly the greatest, success with dithizone determinations has been with colorimetric modifications. Simple colorimetric dithizone determinations developed so far have been of two kinds. They have been described as "one-color" and "mixed-color" methods. In one-color methods developed originally by Fischer and Leopoldi (*10*) for lead and copper, the metal to be determined is extracted from aqueous solution at the proper hydrogen-ion concentration, with an excess of dithizone in chloroform or carbon tetrachloride solution, and the excess dithizone is then washed from the organic solvent by aqueous solutions of weak ammonia, potassium cyanide, or mixtures of the two. Care must be exercised in the control of the hydrogen-ion concentration of the aqueous wash solution. If it is too high, some of the dithizonate in the organic solvent may partially decompose; if it is too low, not all of the excess dithizone will be extracted, especially from chloroform solvent. In other words, there is apt to be a small compensation of errors that must be duplicated in the preparation of standards and in the standardization of solutions. With the equilibrium curves mentioned previously, the removal of excess dithizone could be made more scientifically than can be done by present empirical means. Fischer originally reverted the dithizonates to the equivalent dithizone by treatment with acids and matched the resulting green colors. Others have used the colored dithizonate itself (*30*). The sensitivity of various one-color dithizone methods is approximately 1 microgram, which indicates that analysts using them have been successful in balancing one small error against the other.

In the mixed-color methods (*1*, *6*, *25*), the metals (lead has been determined more extensively than any other metal in this manner) are extracted from the aqueous solutions at optimum pH with an excess of dithizone in chloroform solution. The excess is not removed, but is allowed to partition between the aqueous and solvent fractions and to modify the color of the extracted dithizonate according to the relative amounts of the metal and dithizone. Thus, in the case of lead, a series of colors from green to red may be arranged with intermediate blues, purples, and crimsons; hence the term "mixed colors." The mixed-color method avoids the sources of error of the one-color methods—viz., incomplete removal of excess dithizone or loss of metal to the aqueous phase. The hue of the unknowns is matched against the hues of a standard series of colors. With a little practice analysts become very expert at matching these hues. The sensitivity is greatest at the ends of the series—that is, where the quantity of metal is smallest and the unchanged dithizone is greatest, and just the reverse. Changing the volumes and concentrations of dithizone in chloroform and viewing the extracts through different column lengths produce different ranges of quantities of metal, but each range exhibits the same progression of hues. Very rapid as well as accurate results may be had in the lead-spray-residue field by using Nessler tubes and viewing the colors transversely. The range of lead determined is from 0 to 200 micrograms and the errors are well within 5 per cent. In this way, 1 microgram of lead has been split into 10 distinguishable parts by extracting it with 5 cc. of a dilute dithizone-chloroform

solution and placing the extract in slim 12.5-cm. (5-inch) vials.

Fischer and his associates (*9*) have developed another system of mixed-color colorimetry by duplication rather than by comparison with a standard series. They produce the mixed color in the regular way and then duplicate the hue of the unknown in another vessel of the same shape and containing the same volume of dithizone and adjusted aqueous solution, by alternately shaking and titrating into it a standard metal solution. When the hues match, the volume of the standard solution indicates the amount of metal present. Very accurate results are obtained in this manner on 0 to 10-microgram quantities of metal.

These brief descriptions indicate that excellent results can be obtained with simple apparatus. But the analyst can go the limit in the kind of instruments he wants for measuring the transmission, or for comparing the developed colors. The range can be from Nessler tubes to electrospectrophotometers. In the 16 food inspection laboratories of the U. S. Food and Drug Administration a very moderately priced, home-made, neutral-wedge photometer with a special set of color filters has given most excellent service. The neutral-wedge photometer (*5*) designed by Clifford and Brice is now made commercially. Moderately priced photoelectric photometers using color filters are on the way. A brief description of the principles of photometric measurements as applied to the dithizone colors may, therefore, be of interest.

Fischer and Weyl (*12*) have given the absorption curves of most of the dithizonates and of dithizone in carbon tetrachloride solution. In the United States the transmission curves of lead, mercury, and dithizone in chloroform have been studied. These data give us the information necessary to make photometric measurements of the pure dithizonates with either spectrophotometers, or neutral-wedge photometers supplemented with proper color filters. In one-color methods the maximum absorption of the particular dithizonate is all that is necessary. In two-color methods a wave length is selected where the absorption of the dithizonate is at a maximum and that of dithizone is minimum, or just the reverse. Under these conditions a maximum "spread" is obtained. If Beer's law is obeyed by both the dithizonate and dithizone colors, the transmissions will increase linearly over the range of metal governed by the concentration of the dithizone. That is, if the densities, or simply scale readings, of the colors produced by standard amounts of metal, read through an appropriate cell length, are plotted against metal, a straight line is obtained. This standardization graph of any given batch of dithizone will remain constant if it is kept free from the oxidative effects of light, heat, or oxidizing agents.

One great advantage of photometric measurement is the avoidance of repeated preparation of standards. If curves for various metal ranges, governed by concentration and volume of dithizone and by various cell lengths, are prepared, the analyst need know only the approximate amount of metal in his unknown to fix the concentration of the dithizone solution and cell length necessary, determine the absorption, and read the result from the proper curve. Photometric measurement so far has been restricted mainly to lead, but its success there indicates its rapid spread into the determination of other dithizone metals. One to 100 micrograms of lead (*16*, *18*) have been determined photometrically in biological material with an error of not more than a few per cent.

Purity of Dithizonate Solutions and Accuracy of Results

Dithizone has been criticized for lack of specificity, and all investigators emphasize the importance of separations when interfering metals are present. The value of the dithizone system of analysis could be enhanced from an entirely different direction if the efficiency of separations made under optimum conditions, discussed previously, could be independently tested by some other method not connected with dithizone extraction to determine possible residual impurities. With the exception of silver and mercury, most of the dithizonates are of various shades of red insufficiently distinct to the eye to serve as a means of detection in mixtures; but they do differ from each other to some degree in spectral characteristics.

Optical methods should, therefore, offer some means for distinguishing dithizonates from each other (*26*). Clifford (*4*) has succeeded in reaching this objective in the determination of lead by the use of normal and auxiliary wave lengths or filters. The maximum absorption of the red dithizonates occurs at wave lengths from 500 to 560 mμ (*12*). These maxima are so close together that all the red dithizonates will absorb to some degree at any of these wave lengths and the chance for differentiation at any one selected wave length is slight. However, the definite relationship between the absorption of a given amount of a metal dithizonate at different wave lengths is disturbed by the presence of metal impurities with different spectral characteristics. The auxiliary wave lengths or filters must, of course, be so chosen that the disturbing effect of any interfering metal in any given dithizonate will be at a maximum. If the lead analyst finds, for example, that the data obtained on the normal and auxiliary filters are characteristic of lead dithizonate, he can be certain that his final lead dithizonate is pure, or, at the worst, contains bismuth or tin not to exceed a few per cent of the lead determined. On the other hand, if the checks are not satisfactory, serious amounts of bismuth and/or tin contamination are indicated and a repetition of the lead determination with more careful separations is in order.

The additional assurance of the accuracy of the results when the optical check indicates no impurities is well worth the little extra time required to measure the absorption on one or two auxiliary filters. A recording spectrophotometer should simplify this detection of impurities (*19*). If, in spite of control of hydrogen-ion concentration or complex formation, one or two impurities still persist in appreciable quantity in the dithizonate of the metal being determined, resort can still be had to simultaneous equations based on optical data. How successful such mathematical treatment may be in individual cases remains to be seen. The point to be stressed at the present time is that the introduction of optical transmission or absorption measurements into dithizone investigations may in time furnish a third leg that, with pH control and complex formation, will give a firm support to the dithizone system for the determination of traces of metals.

Conclusion

Up to this time the dithizonates of the metals silver, mercury, copper, lead, zinc, and cadmium have received the most analytical attention. The metals bismuth, tin, thallium, cobalt, nickel, and the noble metals, gold, platinum, and palladium have been considered from the standpoint of interferences in the determination of other metals, but investigations designed to develop methods for their dithizone determinations have not been carried very far. Ferrous iron, manganese, thallic thallium, and indium (*9*) react with dithizone under certain conditions, but their dithizonates are of limited stability and probably of no analytical significance. The field for further investigation is still wide open. Improvements and developments in the dithizone system for the isolation and determination of traces of metals may be expected through (1) hydrogen-ion concentration equilibrium

studies, (2) a greater knowledge of competitive complex formations which will facilitate separations, and (3) photometric transmission measurements of the dithizonate colors which utilize to a greater extent the differences in their spectral behavior.

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Spectrophotometric Methods in Modern Analytical Chemistry

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IN SPITE of the title of this paper, it is not meant to imply that spectrophotometric analysis is a new invention. Sixty-five years ago the first book on the subject by Vierordt (*56*) was published in Germany, but little application of its advantages has been made until recent times. It should, therefore, be proper to review briefly the scope, principles, and advantages of the subject in order to justify its use in place of the simpler methods of ordinary colorimetry; for we must look upon spectrophotometry chiefly as an advanced development in this field.

Definition of Subject and Terms

The term "spectrophotometry" has been defined by a committee of the Optical Society of America (*47*) as the measurement of relative radiant energy as a function of wave length. The energy may come directly from an emitting source or may be transmitted, absorbed, or reflected by absorbing materials. With few exceptions, the analytical chemist is interested in the measurement of light absorbed by a liquid or gas, and it is to this application of the methods that this paper will be chiefly devoted.

It is fair to assume that some readers are not familiar with the precise definition of certain terms commonly used in spectrophotometry. Chemical spectrophotometry has been a kind of no-man's land between chemistry and physics, and a vast entanglement of conflicting designation has grown up which seems more characteristic of the American than of the English or German literature. It is really necessary for any person writing on this subject to define his terms explicitly; so this paper first reviews those which will be used most frequently and by whose use ambiguities may be anticipated. "Colorimetry," for instance, needs no definition for an analytical chemist, yet it is used by the physicist to mean "trichromatic colorimetry," something quite different.

The fundamental relation used in these measurements is the Lambert-Beer's law which is illustrated in Figure 1. It is expressed by this relationship (*53*, p. 18):

$$\log \frac{I_\lambda}{I_{0\lambda}} = -k_\lambda cl$$

Spectrophotometry is a branch of physical chemistry sorely neglected by the analytical chemist. Its advantages lie in the elimination of comparison solutions, the direct calibration of an instrument by a few simple measurements, the ability to determine independently the constituents of a mixture of colored substances, the precise evaluation of the errors of a method, and the extension of measurements to the invisible regions of the spectrum. Speed is an advantage of colorimetric analysis not lost in spectrophotometric analysis. A brief résumé is given of the various types of instruments available, the important errors and limitation of the present methods, and finally examples of the results which may be obtained with actual analytical problems.

where

$\frac{I_\lambda}{I_{0\lambda}}$ = percentage of light transmitted at wave length λ = transmission at wave length λ
k_λ = molecular extinction coefficient for wave length λ
l = thickness of cell (in centimeters)
c = concentration of solution contained in cell (in moles per liter)

For any wave length and any given system the value k should be constant at all dilutions and all thicknesses of absorbent. The constancy of the molecular extinction coefficient is a criterion of adherence to Beer's law. For actually applying this test, a plot of the extinction coefficient against concentration is more convenient. This and certain other terms are defined as follows:

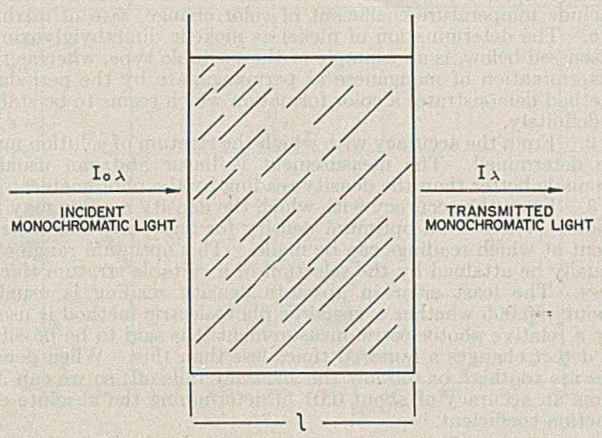

FIGURE 1. CELL CONTAINING LIGHT-ABSORBING MEDIUM

$$D_\lambda \text{ (density)} = \log \frac{I_{0\lambda}}{I_\lambda} = E_\lambda \text{ (extinction)}$$
$$= k_\lambda cl$$
$$\frac{D_\lambda}{l} = k_\lambda c = K \text{ (extinction coefficient)}$$

Only those terms which will be used are included. Density, D, is directly proportional to the concentration of a solution (or gas) and to the cell length, and the substitution of density for the logarithm of the reciprocal transmission eliminates negative signs; so there are real practical advantages in the use of this term. The term extinction, E, used mainly in the German literature, is a synonym. The extinction coefficient is the extinction or density for a centimeter layer of absorbing medium and is directly proportional to the concentration of a solution, if Beer's law holds. K and c are the variables in this equation; k is constant. Beer's law may be valid for colloidal solutions as well as true solutions and some interesting applications of these will be found in the methods referred to at the end of this paper.

Given a method for measuring the density of a solution at a given wave length, we can obtain constant k by measurements on known solutions—the length, l, may be obtained by direct linear measurement. Then, to determine the concentration of an unknown, we measure only the density of a layer of known thickness, and we can calculate the concentration of the solution. It is worth while noting that once a calibration curve is obtained on an instrument further "reference standards" are unnecessary. The number of measurements necessary to obtain a curve may be only one, two, or three, depending on how well the particular method has been investigated, and whether it conforms to Beer's law. The manganese calibration which is shown below is easily reproduced (*44*) and can be obtained with a minimum of measurement. Furthermore, the same straight-line calibration can often be made at high concentrations and extrapolated to low ones. There is no loss in speed over colorimetric methods and, since standards and comparison solutions are generally eliminated, the methods of spectrophotometry are usually faster.

Application of Beer's Law

Using the extinction coefficient as defined in the last equation, really the density of a centimeter layer of solution, and plotting this extinction coefficient against the concentration of a solution, we obtain a straight line as shown in Figure 2.

These values were obtained by measuring, for light of three different wave lengths from the mercury arc, the extinction coefficients of a series of copper sulfate solutions in approximately 1.2 N ethylene diamine. This represents a considerable excess over the amount required to form the violet copper complex with this base. Each straight line represents the extinction curve for a different wave length of light.

In Figure 3 is represented a plot of the logarithm of the molar extinction coefficient against the wave length of light for the same copper ethylene diamine (*34, 50*). The highest point on the curve represents the greatest absorption of the solution and is very close to 546 mμ. The absorption at 578 mμ is slightly less and the absorption at 436 mμ is least of all. Referring to the plot of extinction against concentration,

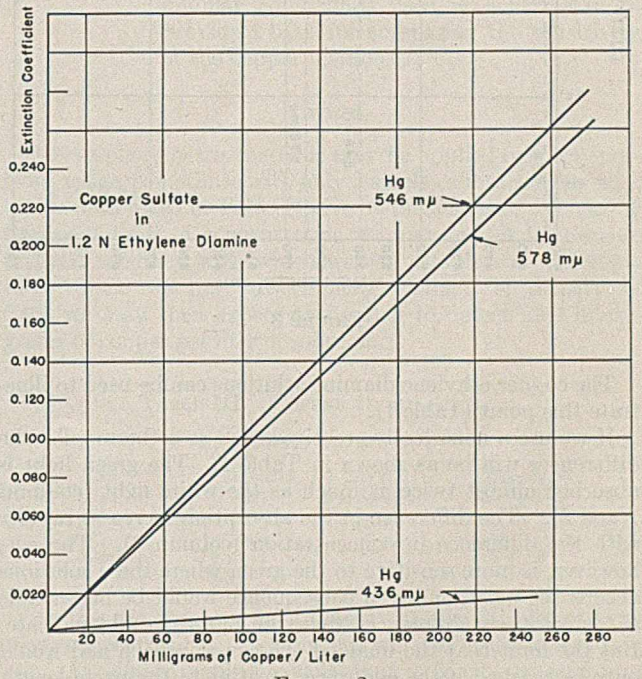

FIGURE 2

it is obvious that we can measure the concentration most accurately when we have the greatest slope to our curves and this is the curve taken at the highest degree of absorption. That this follows from simple mathematical operations can be shown thus:

$$K = kc$$

Differentiating

$$\frac{dK}{dc} = k$$

Or the largest change in density (extinction coefficient) of the solution will be obtained for a given change in concentration, when the extinction coefficient is greatest. This, of course, is at the maximum of the absorption curve.

Visual Sensitivity and Colorimetry

According to the Weber-Fechner Law (*23*), the eye is able to detect a 1 per cent change in brightness except at very high or low intensities. When we wish to determine concentration by changes in brightness level, we should attempt to secure the greatest percentage brightness change for a given change in concentration. In comparing the colors of solutions in ordinary chemical colorimetry, we are using the light transmitted, which is largely derived from the regions of the spectrum where the solutions absorb the least. Consequently the percentage change in light intensity must always be less than when we use the maximum of the absorption band.

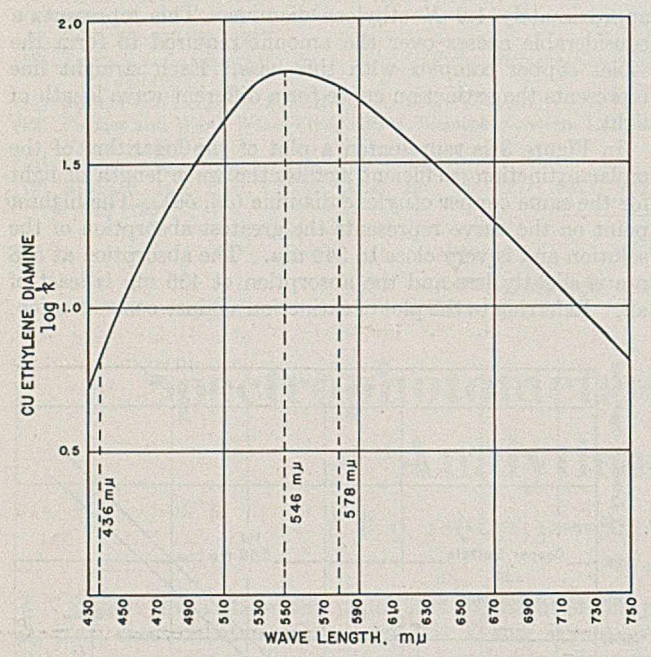

FIGURE 3

The copper ethylene diamine solutions can be used to illustrate this point (Table I).

If we use a nonselective receiver such as a thermopile, the differences will be as shown in Table I. The green light is absorbed almost twice as much as the white light (columns 1 and 2). The difference in the absorption increases rapidly with the difference in concentration (column 3). The eye, however, is more sensitive to the green where these solutions absorb the most, and as a consequence would be better able to detect these differences than the figures would indicate. But the limit that the unaided eye can approach and would only be reached if the eye were sensitive to the wave length 546 mμ alone, is the difference shown when absorption is measured by the mercury green. Of course, the case for colorimetry is much worse if absorption takes place mainly in the violet as with chromate solutions, because here the decreased sensitivity of the eye to violet operates against the discrimination of small differences in intensity. How greatly the sensitivity of the eye decreases in the violet can be seen by a reference to standard values of relative visibility (*27*, *48*). An interesting illustration of this difference is provided by a comparison of three methods for determining copper which will be discussed below.

Evaluation of Errors

This brings us to an important phase of quantitative analysis which does not always receive the attention it deserves, partly, no doubt, because it is not always easy to treat—the evaluation of errors. An important advantage of spectrophotometry over colorimetry lies in the fact that we are able to make very precise comparisons of the most difficult feature in colorimetry—namely, the relative precision and absolute accuracy of determining colors or the degree of absorption of a solution (*54*). In general we may classify the errors of spectrophotometry by saying that they are of three main classes:

1. From the instability of the absorption of the solution. Some colors are stable, others are fugitive. Some may be very readily reproduced, others are never twice alike. All the effects include temperature coefficient of color change, rate of mixing, etc. The determination of nickel as nickelic dimethylglyoxime, discussed below, is an example of the unstable type, whereas the determination of manganese as permanganate by the periodate method demonstrates a color formation which seems to be stable indefinitely.
2. From the accuracy with which the stratum of solution may be determined. The measurement is linear and can usually be made better than the density reading by the photometer.
3. From the accuracy with which the density reading may be made. There is an optimum density for any particular instrument at which readings can be made. This optimum range can usually be attained by the selection of a suitable stratum thickness. The least error in absolute density reading is usually about ±0.005 whether a visual or photoelectric method is used. By a relative photoelectric measurement it is said to be possible to detect changes a hundred times less than this. When densities are too high or too low the accuracy falls off; so we can assume an accuracy of about 0.01 in determining the absolute extinction coefficient.

TABLE I. VISUAL SENSITIVITY

	Light Transmitted		Difference for Heterochromatic and Monochromatic Radiation
	Total white light	546 mμ (green Hg) light	
	%	%	%
1-cm. layer 1.2 *N* in $Et(NH_2)_2$ containing:			
80 mg. copper per liter	91	84	7
240 mg. copper per liter	77	57	20
Difference for threefold change in concentration	14	27	

The manner in which the precision depends on the absolute density for any given form of instrument is illustrated in Figure 4, which shows a plot of the average deviation in parts per hundred against the density measured. These 99 results represent all of one operator's determinations, including the learning period up to the time these figures were tabulated. A Pulfrich photometer was the instrument used and with it the optimum measurable density is predicted at about 1.0 (*61*). An error of 0.01 in density would therefore amount to 1 per cent. That experimental results are in harmony with these findings is evident from this plot of points.

Evaluation of Methods

In Table II is shown a comparison of several colorimetric methods for the determination of copper. The colored compound is indicated in the first column, the wave length at which absorption is determined forms the second column, the change in concentration represented by a change of 0.01 in the extinction coefficient in the third column, and the molecular extinction coefficient in the fourth column. We have already shown that for a given change in concentration the change in density will be greatest for the greatest molecular extinction coefficient. Stated inversely

$$\frac{dc}{dK} = \frac{1}{k}$$

or, in other words, for a measurable difference in density, ΔK, the corresponding change in concentration, Δc, which can be determined will be inversely proportional to the molecular

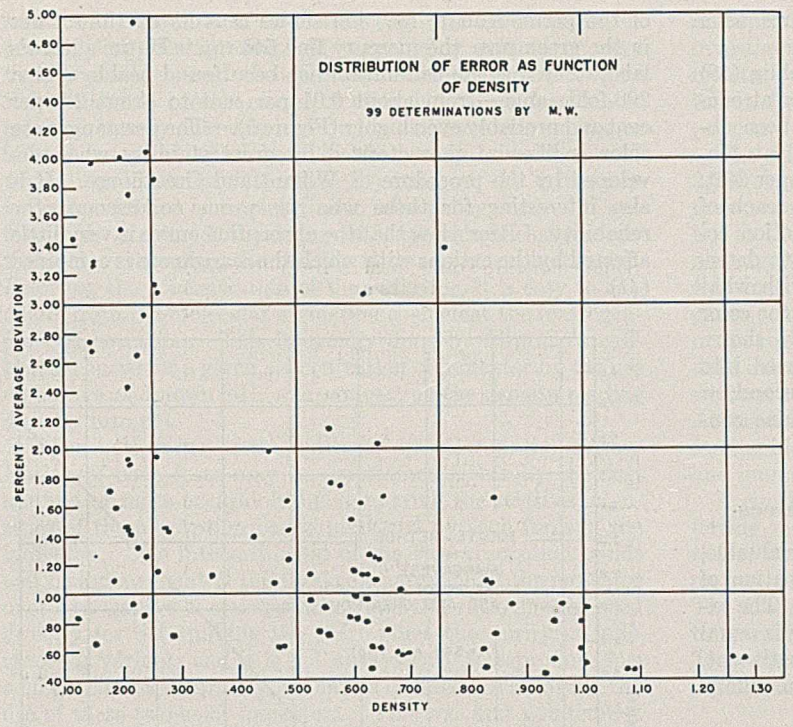

FIGURE 4

extinction coefficient. ΔK in the third column is represented by 0.01 unit of density, or the usual accuracy of a measurement. These data immediately eliminate one of the difficulties of ordinary colorimetry. Usually the relative accuracy of different methods is estimated as a subjective reaction of the author and cannot be quantitatively expressed.

TABLE II. COMPARISON OF METHODS

Substance and Method	Region Absorbed, mμ	Mg./liter $\frac{0.01K}{}$ (1-cm. cell)	$k_\lambda(E)$	Investigation
Copper				
NH_4OH (1.3 *N*)	578 (Hg)	11.6	54.9	S. E. Q. A.[a]
	610		(53.8)	
$Et(NH_2)_2$ (1.2 *N*)	546 (Hg)	9.6	66.5	S. E. Q. A.[a]
HCl (28%)	436 (Hg)	1.23	515.	G. A. Smith[a]
Diethyl-dithiocarbamate[b]	430	0.113	531.	S. E. Q. A.[a]
Dithizonate[b]	508	0.0292	21,800.[c]	Liebhafsky and Winslow (*36*)
Chromium				
Chromate	436 (Hg)	1.7	313.8	Halban and Siedentopf (*20, 32*)
	366 (Hg)	0.12	4,416.	
Iron (ous)				
o-Phenanthroline	508	0.051	11,050.[c]	Fortune and Mellon (*14*)
Manganese				
Permanganate	546 (Hg)	0.23	2420.	W. Murray[a]
	520		2230.	Müller (*44*)
	520		2240.	Mehlig (*38*)

[a] Unpublished data.
[b] In carbon tetrachloride.
[c] Calculated.

For instance, in texts on colorimetry, the hydrochloric acid method for copper is said to be "as good" as the familiar ammonia method. Actually (Table III) it is about ten times as good. The deception arises, as has been earlier pointed out, from the fact that copper in hydrochloric acid is yellow in color—the solution absorbs strongly where the unaided eye is least sensitive. If three solutions of the first three reagents of the concentration indicated, each containing copper in the same concentration (say, 200 parts per million), are compared, the over-all density of the violet and blue solutions will appear much greater than that of the hydrochloric acid solution. But not only may the densities of solutions of markedly different hue be misleading, but even solutions of nearly the same color when their relative intensities are compared.

Weigert (*58*) has described an experiment in which two solutions, both about 0.02 *N* in copper but one 2 *N* and the other 13 *N* in ammonium hydroxide, are compared by daylight in cells 1 cm. thick. The lower concentration of ammonium hydroxide appears darker. When, however, the solution is viewed through a red filter, such as the Corning No. 348 H. R. red shade yellow, the densities are reversed. This phenomenon may be easily explained by an examination of the absorption (*58, 59*) curves of copper sulfate in solutions of various concentrations of ammonium hydroxide, from which it is seen that as the ammonia concentration increases, the absorption of the solution decreases in the shorter wave lengths where the eye is more sensitive and increases in the longer wave lengths where the eye is less sensitive. Although the increase in the red is much greater than the corresponding decrease, the unaided eye does not detect the difference. When, however, these solutions are observed through a red filter which excludes the shorter wave lengths the true state of affairs is immediately apparent.

Also included in Table II are some other familiar compounds used in colorimetry to provide a basis for comparing the sensitivity of the copper methods.

Range

Spectrophotometric methods may be applied over an enormous range, sometimes with only a single calibration curve.

The data listed in Table II give us the means of calculating the lower limit of concentration we may expect to measure and the accuracy with which we can establish a concentration. Since we may discriminate changes of density of 0.01, we may then expect to be able to detect 29.2 micrograms of copper per liter of solution.

TABLE III. COPPER DITHIZONATE

$\left(\frac{\Delta c}{\Delta K} \text{ when } \Delta K = 0.01\right)$

Cell *Cm.*	Capacity *Ml.*	$\Delta c/\Delta K$ *Micrograms/l.*	Copper *Micrograms*
1	3.5	29.2	.....
50	300	0.58	0.18
5 (micro)	1	...	5.8×10^{-3}

By the use of longer cells than 1 cm., we can detect even lower concentrations. A 2-cm. cell will double the lower limit, etc. As cells are regularly available up to 50 cm., we might set 0.58 microgram of copper per liter as the lower limit of concentration we might reach using the larger cells of about 300-ml. capacity. The absolute amount of copper involved would be 0.18 microgram of copper. The least amount of copper which we might determine is by this line of reasoning equal to about 5.8×10^{-3} microgram, since we may work with microcells which require only 1.0 cc. of solution for a 5-cm. stratum. With photoelectric measurements (*53*, p. 84) it is possible to measure densities of 0.001 or 0.0001, thus reducing the measurable amounts by factors of 10 and 100.

But these are largely possibilities, as it is doubtful that anyone has yet achieved anything like these limits in spectrophotometric analysis. Liebhafsky and Winslow (*36*) have pointed out some of the difficulties, largely of a chemical nature, that may be encountered. Kortüm (*31*) has described the instrumental difficulties which must be watched.

We need not be skeptical, however, of these figures as a temporary goal.

At the other extreme of concentration range, Mehlig (*39*) has shown that copper may be determined in mattes at concentrations as high as 20 per cent, and data have been obtained for the determination of nickel in steel (*45*) at concentrations up to 20 per cent with an accuracy of 0.2 per cent. Probably such procedures are entirely beyond the reach of ordinary colorimetry. Calculations based on extinction coefficients of the substances involved may be made to determine when such determinations are feasible, and with what accuracy they may be made. In reactions in which the color develops slowly, Bergami (*4*) and collaborators have shown how an additional parameter time may be introduced into the usual spectrophotometric equations for first, second, or higher order reactions, making it possible to determine concentration during the time the color is developing.

Analysis of Mixtures of Colored Substances

Spectrophotometric methods are exceedingly valuable, in that they are selective and permit the determination of each of a series of colored constituents of a mixture. The extinction coefficient at any wave length for a mixture is equal to the sum of the extinction coefficients for each constituent. By simple algebraic processes (*53*, p. 41) we obtain the following expressions:

$$K_{\lambda_1} = c_1 k_{\lambda_1} + c_2 k'_{\lambda_1}$$
$$K_{\lambda_2} = c_1 k_{\lambda_2} + c_2 k'_{\lambda_2}$$
$$c_1 = \frac{K_{\lambda_1} k'_{\lambda_2} - K_{\lambda_2} k'_{\lambda_1}}{k_{\lambda_1} k'_{\lambda_2} - k_{\lambda_2} k'_{\lambda_1}}$$

A similar expression can be obtained for each concentration. We may measure the molecular extinction coefficients, k, at two different wave lengths, on known solutions. The extinction coefficients, K, can then be measured on the mixture and the individual constituents determined. More than two colored constituents can be determined when the molar extinction coefficients are known for as many wave lengths of light as there are constituents in the mixture. Weigert (*58*) has determined as many as four constituents of a solution by this method. Though the accuracy is not high, it is still usable and is beyond the reach of ordinary colorimetry. Pinsl (*49*, *62*, p. 63) has developed a method for the simultaneous determination of titanium and vanadium in steel by measurement of the absorption of the two mercury lines, 436 mμ and 546 mμ, by a suitably prepared solution.

A simple case of mixed absorption occurs when the molecular extinction coefficient of one constituent approaches zero, but the other constituent absorbs appreciably at some particular wave length. A practically important instance of this type is provided by ferric salt solutions. It is frequently possible to develop a colored compound of manganese, molybdenum, chromium, phosphorus, etc., in a solution of a steel sample, but it is not always easy to compensate by simple colorimetry for the presence of iron. Ferric ions and ferric phosphate, citrate, and fluoride complexes are light yellow in color—mostly because of the absorption of violet light. It is possible, for instance, to form colored nickel complexes in solution with absorption bands where the ferric salts transmit and hence allow the determination of nickel independently of the iron (*45*). The fact that at zero concentration the calibration curve obtained passes through zero extinction indicates that the yellow iron citrate complex is practically 100 per cent transparent at about 530 mμ.

Permanganates show a similarly strong absorption in the green and are another instance when this phenomenon may be useful (*38*, *44*, *45*). A reference to the absorption curve of the permanganate (*32*) ion shows that its maximum lies in the green near the mercury line 546 mμ. In the author's laboratory a single calibration has been found usable over a 200-fold range—from about 0.01 per cent to about 2.0 per cent and probably even higher (Figure 5). The permanganate color is likewise characterized by great stability when developed by the procedure of Willard and Greathouse. It is also interesting for those who are prone to discount the reliability of Beer's law that the absorption curve is very little affected by the cations with which the manganese is combined (*41*).

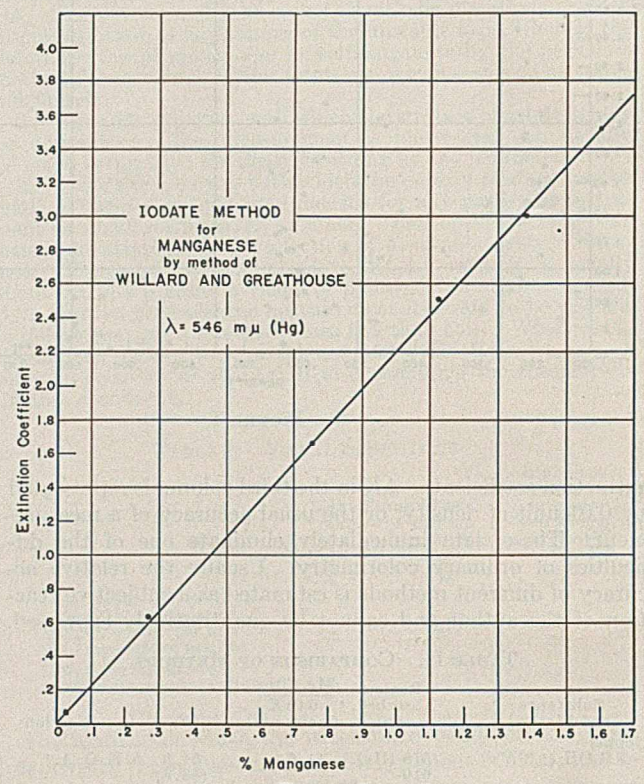

FIGURE 5

Finally, even if we have a mixture of two constituents of the same color, we may, if one is known or remains constant, correct for it or obtain a calibration curve of the type reported by Murray and Ashley (*45*). Phosphorus may be determined in steel by the conversion of phosphoric acid to a phosphovanadomolybdic acid, which possesses a strong yellow color. This reaction may be carried out directly on a nitric acid solution of the steel sample and the calibration curve reveals that at zero concentration of phosphorus the ferric nitrate has a marked absorption. The point at which our calibration curves cut the vertical axis shows the degree of absorption in each case. It can, however, be made to yield results comparable to those of the standard volumetric method using the molybdate complex. This method has not been generally used and has always been considered of low accuracy—mainly because of the difficulty of matching yellow solutions by ordinary colorimetric methods.

Hydrogen-Ion Determination

The determination of hydrogen ions is a special branch of chemical analysis with tremendous practical importance. It is carried out by the use of either potentiometric or indicator methods. Although potentiometric methods have much to recommend them from convenience, they are subject to some very serious limitations especially when used on unbuffered

solutions. The use of indicators, on the other hand, is generally applicable except in very highly colored or very turbid solutions, and the spectrophotometer doubtless provides the most precise means of using indicators. Indicator action is usually characterized by the existence of a substance in two forms, either or both of which may be colored, and whose equilibrium relationship depends on the hydrogen-ion concentration of the solution in which they occur. If one form only is colored, the degree of conversion is easily ascertained by measuring the density with a spectrophotometer. Knowing the concentration of the indicator, it is easy to calculate from the molecular extinction coefficient the hydrogen-ion concentration. This is usually done by forming a calibration curve for a given concentration of indicator by the use of buffers of known pH, and converting the density reading directly into pH.

When both forms of the indicator are colored, the concentration of each form may be ascertained either by the first method or more accurately by comparing the relative intensities of the absorption bands produced by each form of the indicator. The intensity ratio of the two absorption bands will be independent of the total concentration of the indicator over wide ranges of concentration, and this ratio may be used directly for determining the pH. Since the purity of indicators is variable and it is not always easy to reproduce low concentrations accurately, we have a decided advantage in the use of these two-color indicators. This was first pointed out by Holmes (*25*) in 1924, and since then has been applied by several investigators. Brode (*6*) and Fortune and Mellon (*15*) have published interesting and useful families of curves showing the growth and decay of these absorption bands as the pH is changed. Hähnel (*19*) has recently shown that a linear relationship exists between the logarithm of the ratio of the intensity of these bands and the pH.

Spectrophotometry in the Infrared and Ultraviolet

So far this discussion has been implicitly applied only to materials absorbing in the visible spectrum. Actually, materials absorbing in the infrared and ultraviolet may also be determined by the principles already outlined, but the technique involved is considerably different for each. Although many successful specific applications have been made, this review must be confined to a few interesting cases merely to demonstrate their usefulness. Many of these invisible absorption bands are of extraordinary intensity and may be more characteristic than the color of permanganate.

Carbon dioxide is one of these substances and McAlister (*37*) of the Smithsonian Institution has constructed an apparatus for the continuous determination of carbon dioxide in plant respiration experiments based on this principle. It depends on measuring the degree of absorption of a band at 4,200 to 4,300 mμ in the infrared which is specific among gases for carbon dioxide. The band is isolated by a spectrograph serving as monochromator and the degree of absorption of the radiation is determined by a thermopile. This arrangement allows the determination of 1 p. p. m. of carbon dioxide in about 5 seconds. The period of the galvanometer is the limitation on speed and can be greatly decreased. This represents a percentage of 0.0002 to 0.0005 per cent of carbon dioxide per scale division. Similarly Warburg and Leithauser (*57*) determined ozone, nitrous oxide, and nitrogen pentoxide in the presence of one another and nitrous oxide in the presence of nitrogen tetroxide. Ammonia, water vapor, chloroform, and many other vapors show strong infrared absorption which might be used for their determination.

Benedict and collaborators (*3*) have carried out the analysis of deuteromethanes and ethanes by means of infrared absorption spectra. Solutions are also open to infrared investigation. Errera (*11*) has recently determined traces of moisture in other solvents by the absorption of a band attributed to dissociated water in concentrations less than 0.050 per cent.

The detection of mercury vapor by its absorption of the 2,537 Å. resonance line from a suitable mercury lamp is the most outstanding commercial application of spectrophotometry in the ultraviolet region of the spectrum. One part of mercury vapor in 100,000,000 parts of air is the sensitivity of this instrument (*1*). Carr (*7*) reports that among the organic compounds benzene is as easily detected in the Schumann region at 1,732–89 Å. as mercury. Benzene cannot be used even to wash stopcocks in the supplementary apparatus without leaving traces that can be identified for days after. Absorption spectrophotometry has been used in various ways for the identification and determination of vitamins A and C and innumerable other organic products (*53*, p. 26).

A considerable advantage could often be gained by transferring measurements usually made in the visible region of the spectrum into the ultraviolet. The well-known colorimetric determination of chromium is an example of this. The absorption of chromates rises steeply, going from violet to ultraviolet, and is at a maximum at about 366 mμ (*33*). The actual advantage to be gained by a change from 436 mμ to 366 mμ is a fourteen-fold increase in absorption.

Construction of Instruments

The development of the usefulness of the spectrophotometer has been a direct result of the efforts of the instrument maker. Even to mention all the instruments made would be extremely difficult; this paper merely discusses a few that are typical to illustrate the means of operation. First, however, a little of the theory involved in the measurements will be covered.

As the name implies, the spectrophotometer does two things—it selects a portion of the spectrum, and then measures the intensity of the light selected by means of a photometer. Practically, we cannot usually do better than to select as narrow a band of wave lengths as possible. In general, there are two methods in use for doing this—by filters, or by a monochromator. If either is used with a continuous source of light, the purity of the light separated is not very great, but the monochromator gives purer radiation—that is, a narrower band of wave lengths—than the filters. The band of radiation from the filters may be 250 Å. in width and less. Usually the purity of the spectrum entails a sacrifice in the intensity of light transmitted, and one must arrive at a compromise of these two factors. By using a discontinuous source such as a mercury arc and suitable filters or monochromator, light of high intensity and high purity may be obtained for the lines available (*47*, p. 185). However, the lines are not always where you want them, and the arc has a background which detracts from the purity of the light. The lines are frequently complex and it is only the exceptional lines which attain a true monochromatic purity.

It is customary to call photometers with a system of filters "absolute colorimeters," and the measurements made with them "abridged spectrophotometry" in order to differentiate them from the instruments employing prism monochromators which give more accurate results. Since it is merely a difference in the breadth of the band which is taken for a determination, the distinction appears artificial. The fact that a selected portion of the transmitted light is examined is the essential difference between the spectrophotometer and the colorimeter, and the instrument with filters consequently is more like a spectrophotometer than a colorimeter. Probably no one will question that the results obtained with the

simpler forms of instrument can be done at least as well with the more complex. Most of the absorption bands with which we deal are broad, such as those of chromates and permanganates, and if the measurement is made at the peak of a band, excellent results may be obtained. If, however, the measurement must be made at the edge of a band where the absorption is changing rapidly, the different portions of the light transmitted by the filter are absorbed to a different degree with the result shown in Figure 6.

The dotted line represents the transmission of a violet filter with a peak transmission at 430 mμ. The solid lines represent the absorption of a yellow solution at different concentrations. Since the lines cross the filter unsymmetrically, the chromaticity or color of the light through the filter varies and makes matching very difficult or impossible. In such cases Beer's law will not hold for the measurements. This effect may be apparent to a lesser degree with a monochromator, depending on the slit widths used.

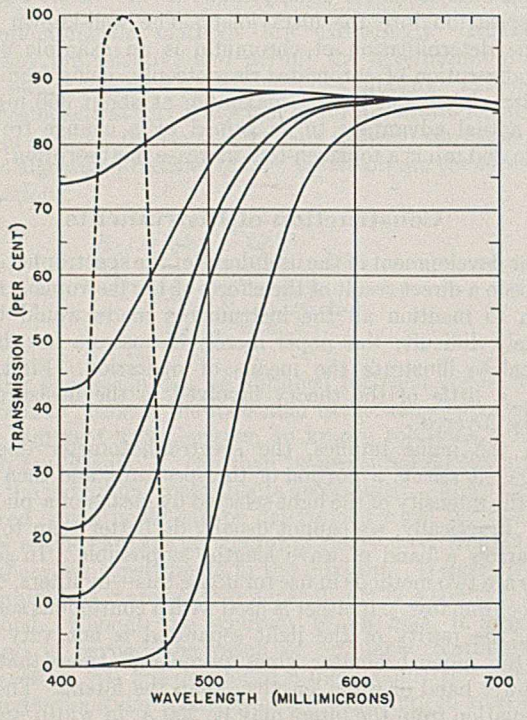

FIGURE 6

Solid filters are usually of glass or gelatin. The gelatin filters almost invariably show a high transmission to the longer wave lengths beyond 7,000 Å., which can be blocked by the use of suitable glass filters or a copper sulfate solution. Although colored glasses of various sorts are widely manufactured, almost the only ones suitable for the purposes discussed above are the Corning glass filters (*9*), and the Jena glass filters (*12*) supplied through the Fish-Schurman Corporation. The Eastman Kodak Company (*10*) markets a large number of filters, almost all of which are of gelatin mounted in glass. These manufacturers all supply literature describing the stability, transmission curves, etc., of their filters. An unusual combination for 560 mμ is that by Gibson (*17*).

Liquid filters may be employed in some instruments and may be prepared according to a large number of recipes (*5, 30, 59*).

It is frequently possible to devise filters for specific purposes by a reference to the Atlas of Wood and Uhler, or absorption curves in such tables as the International Critical Tables or the Physikalisch-Chemische Tabellen of Landolt-Börnstein.

It is not possible to describe the various forms of monochromators in detail, but one may summarize them by saying that they operate on the principle of the dispersion of light as does the spectrograph. The materials of which they consist depend on the region of the spectrum in which they are expected to operate, and the degree of performance that is expected of them. An interesting application of chromatic polarization in a monochromator is described by Öhman (*46*), though it has not come into any general use.

The photometer for the visual region of the spectrum may consist of a differential device adjusted by the eye, or a thermopile, photoelectric receiver, or photographic arrangement which may also be used in the ultraviolet or infrared region of the spectrum. It is not possible to do more than review briefly the methods used in the visible spectrum as being typical of the technique involved. Discussions of infrared methods will be found in a paper by Barnes and Bonner (*2*) which appeared last year. Kortüm (*31*) also gives a useful review of the methods and difficulties involved in absorption spectrophotometry in the ultraviolet. An interesting instrument has been constructed by Hogness and his co-workers (*24*) at the University of Chicago.

The various forms of photometers commercially available will be of most interest to the chemist who is going to use spectrophotometry as a tool in other work.

Some of the many forms of photometers have been described by Gibson (*13, 16*), Weigert (*59*), Mellon (*40*), and Twyman and Allsopp (*53*). A short summary of instruments adapted to the methods discussed in this paper will be given in order to illustrate the different types of instrument available. Further details can be obtained from the individual manufacturers.

The Zeiss Pulfrich photometer, the progenitor of the "absolute" colorimetric type of instrument, has been frequently described in the literature. It usually employs two absorption cells, in one of which is placed the material investigated and in the other a blank. Two beams of light of equal intensity from an incandescent lamp pass through the cells and may be observed simultaneously through an eyepiece as a circular divided field. Twelve filters contained in the eyepiece enable one to isolate bands about 25 mμ wide at distributed intervals through the visible spectrum. The absorption of the unknown is compensated by reducing the intensity of the unabsorbed beam by means of a variable aperture in the objective. When the two beams are of equal intensity, the amount of absorption can be read from a calibrated drum either as extinction or percentage transmission. A mercury lamp may also be used in conjunction with special filters and the three wave lengths 436 mμ, 546 mμ, and 578 mμ can be separated.

The Leitz instrument "Leifo" is similar in equipment but uses polarizing prisms to vary the intensity of the light instead of apertures. The Aminco wedge photometer is also similar to these instruments but varies the intensity of light by means of a calibrated neutral wedge. The Bausch & Lomb spectrophotometer employs an elaborate arrangement of crossed Nicol prisms for varying the intensity of the two light beams. The two beams pass from the photometer through the monochromator which takes the place of the filter and may be adjusted to give a narrow band of wave lengths at any part of the spectrum. In principle this instrument is similar to the König-Martens and Hilger-Nutting spectrophotometers. These instruments are the most highly developed of those employing visual adjustment.

Instruments employing photoelectric or barrier-layer cells instead of the eye have come into general use and almost every chemical supply house has one of these on the market. Though the simpler forms of these instruments are not usually called spectrophotometers, they can be converted into a cruder form of the instrument by the use of filters. Gibson has pointed out (*16*) that the name "colorimeter" is just as inappropriate except under very special conditions.

The photelometer, an instrument manufactured by the Central Scientific Company, shows the use of a barrier-layer cell to read light intensity directly. It consists principally of a lamp, variable diaphragm, lens, absorption cell, filter and barrier-layer cell,

and microammeter. Readings are taken with and without solution under investigation in the absorption cell and the concentration is determined by calibrating the meter reading with the percentage composition.

Differential instruments employ two barrier-layer or photoelectric cells illuminated by a common light source and balanced to zero current against one another with and without an absorbing solution in place by changing the light on one cell or by a slide wire bridge. Instruments of this type are the Lange Universal photoelectric colorimeter, the KWSZ photometer, the Hilger absorptiometer, and the Aminco photometer.

Finally, the General Electric Hardy recording spectrophotometer comprises an entirely automatically operated monochromator and photometer with a recording mechanism which draws a graph of transmission *vs.* wave length between 400 mμ and 700 mμ. Very complete descriptions of this instrument are given by Michaelson (*42*, *43*), Hardy (*21*, *22*), and Gibson (*18*).

Reactions and Methods

It follows from what has been said that any colorimetric method can be used for the methods described. Excellent compendia of these have been provided by Yoe (*60*) and Snell (*51*) and specialized books have appeared (*55*, *62*, *63*), and descriptions of clinical methods (*63*). Potential material can easily be found by an inspection of the spectral absorption curves in International Critical Tables, Landolt-Börnstein's "Physikalisch-Chemische Tabellen," Coblentz's "Investigations of Infra-Red Spectra" (*8*), and LeComte's "Le Spectre Infrarouge" (*35*). Many useful reactions are described by Strafford (*52*) and in the recent "Tables of Reagents for Inorganic Analysis" (*26*). Werner complexes because of their nonionic character are especially adapted to the methods discussed but have been exploited to only a limited degree. The *o*-phenanthroline ferrous complex mentioned in Table II is an example of this type of compound. Its stability and indifference to large changes in solution environment are demonstrated by Fortune and Mellon (*14*).

The density of many colloidal solutions may be photometered and, when their adherence to Beer's law is not sufficiently close, calibration curves can be constructed to convert densities into concentrations. The determination of low concentrations of phosphorus in steel by the development of a turbid solution of strychnine-phosphomolybdate has been worked out by Koch (*29*, *62*), and the determination of nickel, copper, and cadmium by Juza and Langheim (*28*).

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Present Status of Colorimetry

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In reviewing the present status of colorimetry attention is directed especially to the importance of colorimetric methods of measurement as revealed in current periodicals and two comprehensive treatises; the variety of applications already made, together with the general limitations of such measurements; and a classification of the kinds of methods available for making colorimetric measurements, including suggestions for a consistent usage of terms.

IN THE current literature of optics and analytical chemistry the term "colorimetry" may have quite different meanings for different individuals. To the physicist it implies measurement of color in the sense of determining the magnitude of the three attributes, hue, brightness, and purity, or of the tristimulus values, red, green, and violet (*48*). His interest might be considered as color analysis. To most chemists colorimetry generally implies measurement of the amount of a constituent by comparison of the colored system containing the unknown with a similar system containing a known amount of the desired constituent, or with a system visually equivalent to the latter. In this sense the chemist's colorimeters are really only comparators. There are, of course, other methods of measurement.

It is the author's hope that this presentation will serve to correlate, at least from the viewpoint of analysts, the interests of both the physicist and the chemist in the problem of the measurement of colored systems. Limitations of space restrict the material to little more than an outline of the items concerned.

The Domain of Colorimetry

In its broadest sense colorimetry includes all procedures which have as their objective the measurement of some property related to the color of the sample. Systems possessing the attributes of color comprise everything we recognize as being colored, including solids, liquids, and gases. The outline of methods of measurement given below provides for all such cases. Since the chemist, or at least the analyst, has been most concerned with applying colorimetry to solutions, this discussion is confined largely to systems in the liquid state. In addition, it is limited to quantitative work, as a previous paper (*32*) covered the general analytical uses of color.

In solutions the solute may be considered as the desired constituent. If such a constituent is to be determined colorimetrically, either it must itself possess suitable colorimetric characteristics, such as those of the permanganate ion, or, as much more frequently happens, it must be capable of reacting with some reagent to give a substance having suitable colorimetric characteristics, such as those produced by an aqueous solution of chlorine reacting with *o*-tolidine.

The extent to which quantitative methods have been based on the colorimetric properties of such systems is shown in the treatises of Yoe (*53*) and of Snell and Snell (*41*). The latter work, comprising two large volumes, includes over 900 methods in which approximately 700 different reagents are used for nearly 400 elements, radicals, and compounds (*40*). Much material of general interest concerning colorimetric methods of analysis is summarized in these treatises. In 1936, for the first time, a special section of the *Annual Reports of the Chemical Society* (*43*) was devoted to colorimetry. Many papers are appearing each year to increase our knowledge of this field. The dates of the references cited here reflect the present interest in such work. Incidentally, this wealth of material seems to justify the conclusion that the time has arrived for elementary books in quantitative chemical analysis to divert a little attention from gravimetry and titrimetry to colorimetry.

The range of sensitivity of the methods is highly variable. It is not possible to generalize, because there are methods which are sensitive to one part per billion (an exception, of course), and others applicable only to 10 to 20 parts per million (also an exception). Most methods are applicable over a range from about 0.5 to 10 p. p. m. The range of application is determined by the intensity of the color of the system to be measured and by the sensitivity of the means of measurement used. With very low and with high concentrations of the desired constituent small differences in amounts cannot be determined reliably. Such methods usually are not applicable, without dilution, to quantities greater than 1 per cent of the total sample, although, using a spectrophotometer, Mehlig worked with much larger amounts (*28*). The absolute accuracy differs for different methods of measurement. With visual comparators it is usually within 5 per cent. Well-designed photoelectric instruments, on account of their increased sensitivity, give a somewhat lower error.

Colorimetric measurements can be applied to a wide variety of systems. In recent years there has been a large number of papers in biochemistry on the application of such procedures, with methods for the clinical laboratory predominating. However, one finds colorimetric methods applied to industrial products ranging from foods to steels—in water analysis, for example, they have long been official for certain constituents.

Methods of Measurement

Many chemists are probably not fully aware of the number and the variety of devices which have been proposed for measuring different characteristics of colored systems. The last two decades have brought notable advances in the introduction and improvement of such instruments. All the apparatus is based essentially on the measurement of a systemic property (*31*).

The résumé following is intended to be an outline of the most important kinds of instruments now being used for colorimetric measurements in analytical work. The classification is a modification of that presented recently by Gibson (*13*) in reviewing the subject from the viewpoint of physics. Specific commercial instruments are mentioned as illustrative of a class rather than as necessarily the best of the type available.

Since the term "colorimeter" is not used in the same sense in physics and chemistry, the author suggests that it be reserved in each field for any instrument that measures a property that is a function of one or more of the attributes of color. Then instruments which have a special application can be designated by more specific terms, as indicated below. Used in the sense proposed, colorimeters would include

spectrophotometers measuring the visual spectrum but not those for the ultraviolet or infrared regions. This is analogous to limiting the term "light" to the visible region, ultraviolet "light" then being a misnomer.

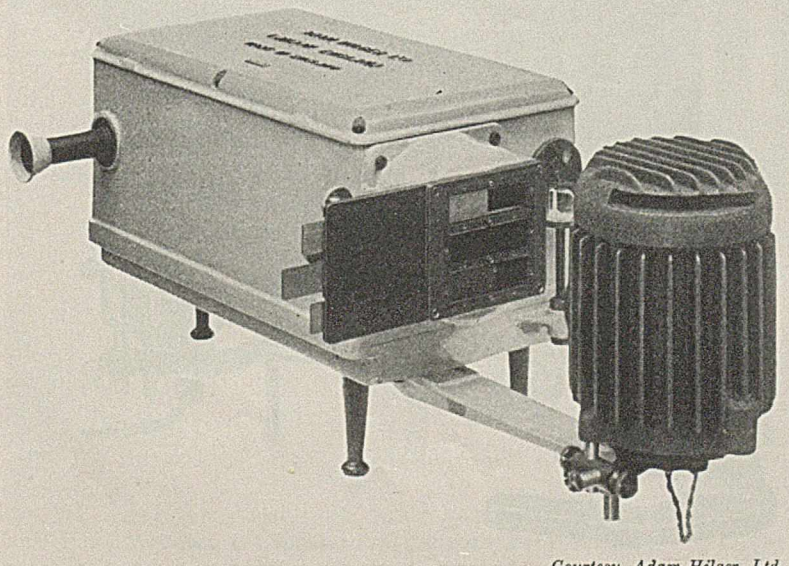
Stimulimeters

The instruments called colorimeters by physicists will be considered first. Since such apparatus is designed to match, by means of a suitable combination of known stimuli, the stimulus of the system measured, the term "stimulimeter" seems appropriate. All students of optics are familiar with the matching of a given color by means of three rotating disks, colored red, green, and violet, so arranged that the relative amounts of the three "primaries," or stimuli, can be varied until a match is obtained. Different modifications of such devices depend upon the nature of, and the method of combining, the stimuli. (Since it seems probable that one might have stimulimeters, comparators, and absorptometers in other scientific fields, it may be desirable to use the word "color" with each term. We would then have color stimulimeters, color comparators, and color absorptometers.)

In the additive type the observer mixes the primaries or standards in such a manner that the mixture is the sum of the components. Examples of those dependent upon material color standards are the apparatus using Munsell paper disks, and the instruments designed by Donaldson (*6*), Guild (*17*), and Newhall (*35*). In those using spectrum primaries we may distinguish between the trichromatic type in which light of three different wave lengths is mixed, as in the apparatus of Guild (*18*), Verbeek (*49*), and Wright (*52*), and the monochromatic type in which light from a heterogeneous stimulus ("white light") and a small wave length band of the spectrum are mixed. For purples the heterogeneous stimulus is matched by adding the spectrum light to the sample light. The apparatus designed by Priest (*37*) is an example.

In subtractive instruments the illuminant is passed successively through the standards, each of which absorbs in turn part of the light transmitted by the previous one, until a match is obtained. The standards may be such materials as solutions (*4*), wedges of dyed gelatin (*22*) or glass (*23*), or glass disks as used in the Lovibond tintometer (*47*).

Of the various stimulimeters the Lovibond instrument is apparently the only one used much by chemists. Even its use is for color specification rather than for chemical analysis, although it is possible to correlate concentration and certain stimulimeter readings. In addition to the difficulty of reproducing the arbitrary filters and of relating their tristimulus values to concentration of solute in a colored system, more important difficulties have prevented general adoption of such apparatus, especially the additive type. Some of the instruments are too complicated for routine work and in all of them the quality of the light source must be carefully maintained and the observer should have both a normal visibility curve and normal mixture data (*20*). Further information concerning these instruments may be found in treatises on physics or in Guild's papers (*15*, *16*).



Courtesy, Adam Hilger, Ltd.

FIGURE 1. DONALDSON TRICHROMATIC STIMULIMETER

Comparators

In the instruments which should be called comparators the measurement is made by matching the system containing the desired constituent in unknown amount with a similar, standard system containing the desired constituent in known amount, or with something visually equivalent to such a standard. When the desired constituent itself is suitable for preparing the standard, the solution is prepared in permanent form whenever possible. In other cases, in order to allow for compensation of errors, it is necessary to prepare the standard at the same time and under the same conditions as the unknown.

Visually equivalent standards, many of which are permanent, have been prepared from various materials, such as solutions of inorganic or organic compounds, glass, and even color cards. Ordinarily they are recommended for the best work only when it is impossible or inconvenient to use the desired constituent itself for standards. Their spectral transmission (or reflection) characteristics are seldom the same as those of the desired constituent, which renders matching impossible under illuminants of different spectral distributions. The spectrophotometer has revealed (*7*, *29*, *44*) notable differences in transmittancy in some permanent standards and unknowns matching them visually under a given illuminant.

Among the many devices which have been made in developing apparatus to be used as comparators three types may be recognized.

STANDARD SERIES TYPE. The old standard series technique remains probably the simplest and least expensive of the widely used colorimetric methods. It is especially desirable for solutions not obeying Beer's law, since the unknown and standards have the same thickness. Solutions or glasses are used most as standards, the matching being accomplished visually. The new roulette comparators facilitate comparison in Nessler tubes, preferably with plane glass bottoms.

BALANCING TYPE. Another widely used method consists in comparing the unknown solution with a single standard solution, the depth of one being fixed and that of the other changed until a match is obtained. If the systems obey Beer's law, the concentrations of the desired constituent in the two solutions are inversely proportional to the depths of solution measured. For the many systems not obeying Beer's law the two solutions must have nearly the same concentration, or the necessary correction must be determined experimentally.

The Duboscq comparator is the best known type of instrument used for balancing. Snell and Snell (*41*) and Yoe (*53*) illustrate a number of the many variations of design used, practically all made for visual matching. Recently Goudsmit and Summerson (*14*) made an interesting modification in which the matching is accomplished by means of two photocells.

Thiel (*46*) has proposed a method which he designates as "absolute colorimetry," based upon determining the specific extinction of the unknown by matching the solution with a gray solution prepared from dyes and having a specific extinction of 0.5. Thiel has published more than fifteen papers on the application of the procedure.

DILUTION AND DUPLICATION TYPE. Another type of comparator is based upon matching the known and unknown solutions by means of dilution or duplication of the color. Kolthoff and Sandell (*25*) refer to duplication as colori-

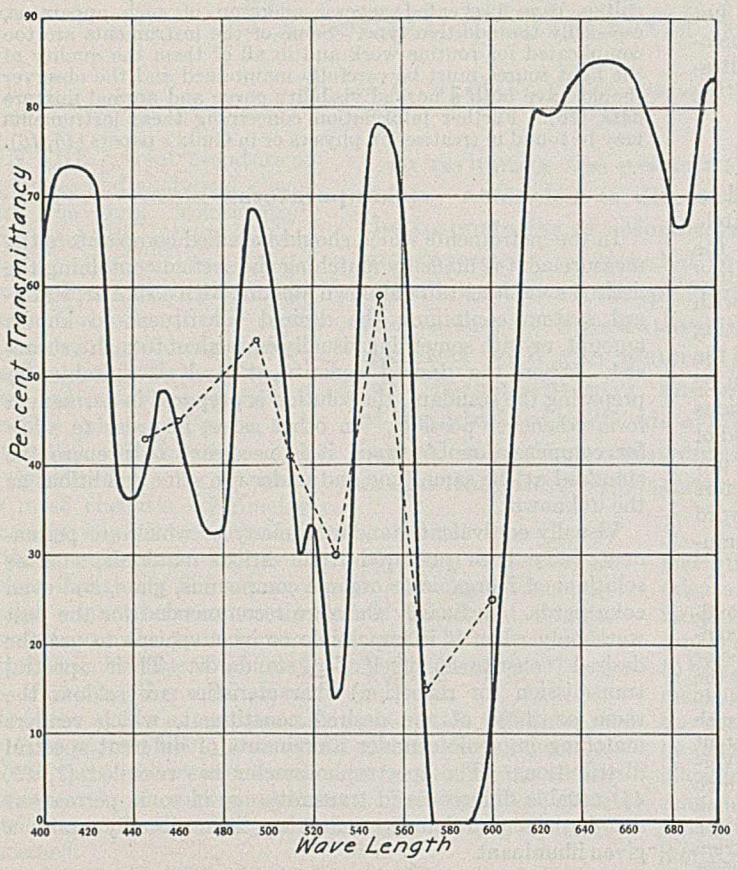


FIGURE 2. SPECTRAL TRANSMISSION CURVES FOR A DIDYMIUM GLASS

Solid curve, with a recording spectrophotometer
Broken curve, with a filter photometer

metric titration. Although there are commercial devices for this operation, their general usefulness is such that they will not be considered further here. Details may be found in the treatises mentioned above. Matching in Nessler tubes by duplication is recommended in some steel laboratories.

Absorptometers

The third class of instruments comprises devices which may seem to be too different in nature to justify inclusion in the same group. Essentially, however, they all measure, for a given illuminant, the light-absorptive capacity of the system studied. Since absorption is the property measured, the instruments may be called absorptometers. (For some years the Kipp and Zonen Co. has used the term "absorptiometer," and recently Adam Hilger has adopted it. The spelling "absorptometer" seems preferable, to be consistent with "reflectometer," a term currently used.) Instead of being graduated in terms of absorption, the conventional practice is to use transmission (or something related to it, such as extinction coefficient) for transparent media and reflection for opaque material. Appropriate names for the instruments are transmissimeters and reflectometers, respectively.

FILTER PHOTOMETERS. In general, photometers are designed to measure intensity (brightness) of illumination. Those used in colorimetry measure the proportion of light incident upon a system that is transmitted (or reflected). Since all colored solutions absorb some of the incident light, the analyst's problem is to relate the concentration of the desired constituent to the amount of light transmitted.

The solutes in colored solutions absorb light in certain definite regions or bands in the visible spectrum. The variation in transmission with concentration is greatest when the incident light is restricted to the spectral region of the solute's greatest absorption. In photometers this is accomplished, at least partially, by interposing a suitable filter, usually of glass, between the illuminant and the observer, hence, the name filter photometer. Several manufacturers have selected a series of 8 or 9 glasses for which the regions of maximum transmission are fairly well spaced from 450 to 650 mμ. Such a filter permits the passage of a wide spectral band of light. Selection of the best filter for a given determination should be based on the spectral transmission curve of the solution to be measured. Thus the absorption band of the permanganate ion corresponds with the region of maximum transmission of a signal green glass. Manufacturers recommend a specific glass for a given determination. Where the measurement justifies its use, a monochromatic illuminant, such as a particular line of the mercury arc, may be used.

The actual quantity measured depends upon the instrument. Some are designed to give directly the percentage of incident light transmitted; others have arbitrary scales or are read in terms of some units which must be converted to the amount of the desired constituent. Generally a curve is constructed, from a series of standard solutions, which coordinates concentration of the desired constituent and the corresponding read-

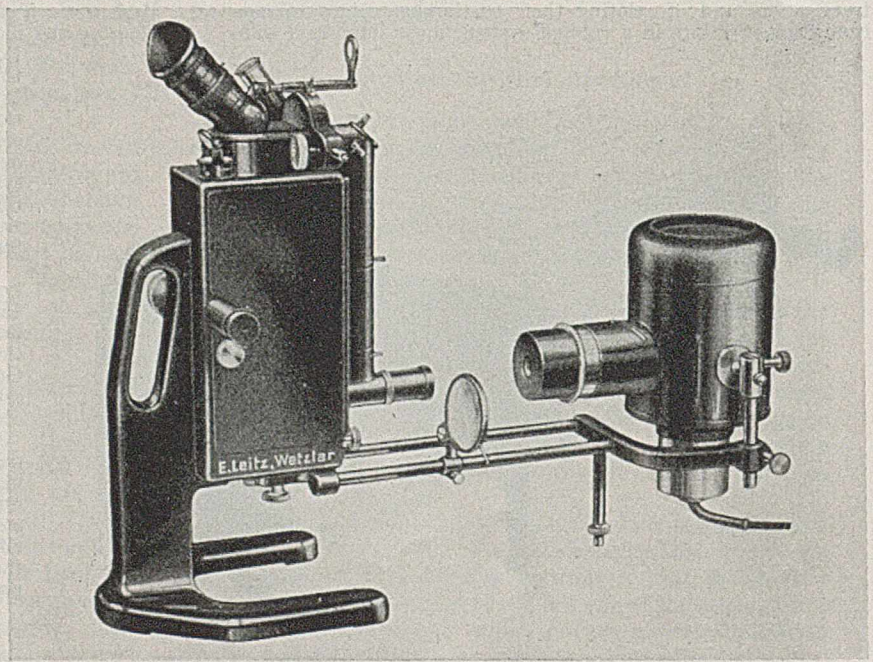


FIGURE 3. LEIFO PHOTOMETER

ing of the instrument. If the solution obeys Beer's law, one may apply the Bouguer-Beer equation

$$c = \frac{\log I_0/I}{el}$$

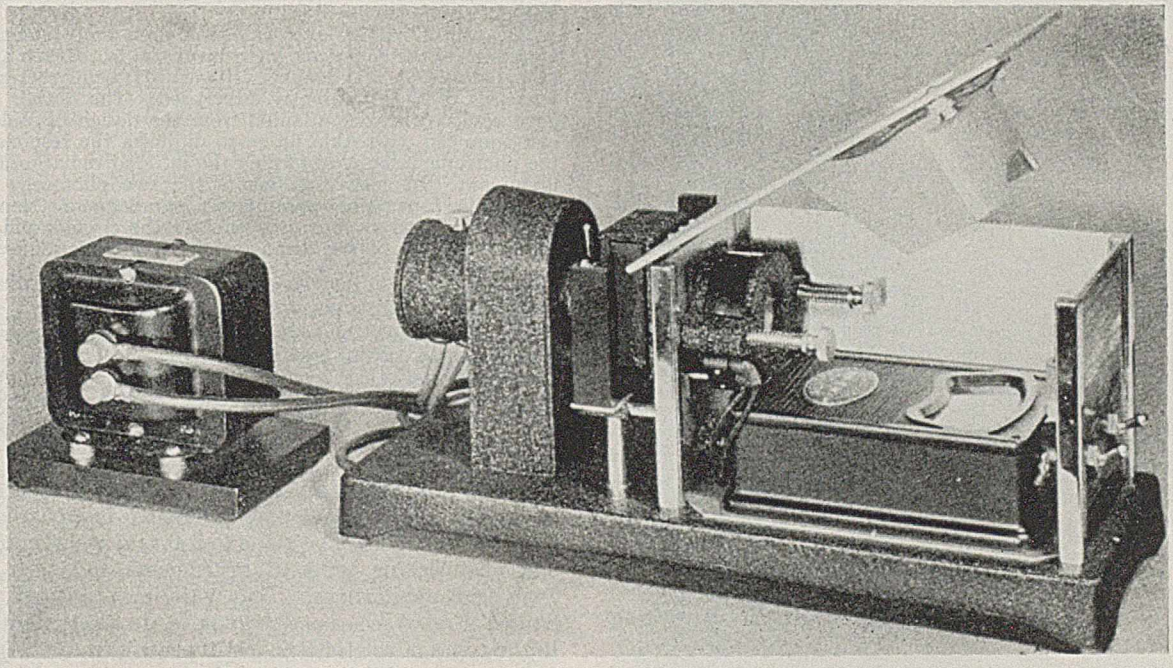
in which c = concentration (in moles per liter), l = thickness of the solution (in cm.), I_0 = intensity of the incident light, I = intensity of the transmitted light, and e = molecular extinction coefficient. On plotting the determined transmittancies for a series of solutions on a logarithmic axis against the corresponding concentrations on an equal division axis a straight line is obtained. Since a given cell thickness is used and the slope of the curve is unimportant (as long as suitable sensitivity is obtained), it is necessary only to determine the transmittancy for a known solution and draw a straight line through the determined point to 100 per cent at zero concentration. If the solution does not obey Beer's law, as many do not, a curve must be constructed for a series of different concentrations of the desired constituent. Once the curve is established in either case there is no further use for standards.

Neither filter photometers nor comparators yield a fundamental color specification, since they do not really measure color as such nor provide data for calculating color stimuli. These instruments come nearest, perhaps, to measuring relative brightness. For certain solutions Keane and Brice (*24*) have proposed determining a "color index" with their instrument from the formula $100 - 100\,G/R$, G and R being the measured transmittancies of the solution for the respective filters.

Although some analytical writers have referred to filter photometers as spectrophotometers, such an instrument can be considered at best as nothing more than an abridged spectrophotometer. If there are eight filters, the transmittancy of the sample can be determined for each one. On account of the width of the spectral band passed by each filter, these eight points cannot establish a reliable spectral transmission curve for materials having steep absorption bands. The broken line in Figure 2 is drawn through the points obtained on such an instrument (data provided by the manufacturer of the instrument), the wave lengths being those given by the manufacturer as the medium wave length for the filter. The smooth curve gives the spectrophotometric data for the same sample for a band width of 5 mμ.

On account of the differences in their construction, it is convenient to consider visual and photoelectric photometers separately.

Visual Type. In the Pulfrich photometer (*38*), as manufactured by Carl Zeiss, Inc., two light beams enter the optical system, one passing through the solvent only and the other through the solution. The observer brings the two halves of the optical field to a match and determines the magnitude of the absorption from the readings on the micrometer drum heads. A series of eight glass filters provides for isolating spectral bands approximately 20 to 25 mμ wide. In recent years many papers have described the use of this instrument as a means of determining



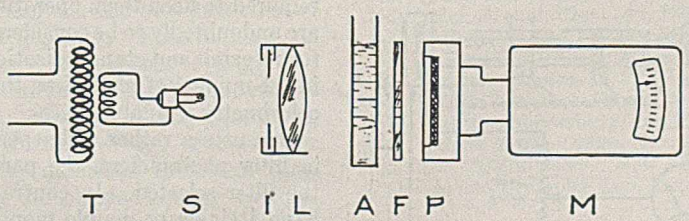


FIGURE 4. SHEARD AND SANFORD PHOTOMETER

T. Constant wattage transformer
S. Light source
I. Iris diaphragm
L. Lens
A. Absorption cell
F. Light filter
P. Photoelectric cell
M. Microammeter

concentration of solutions, and for a variety of other optical purposes.

A more recent instrument of the same general type, illustrated in Figure 3, was introduced by E. Leitz, Inc., as the Leifo photometer (*27*). The relative intensity of the two light beams is varied by rotation of polarizing prisms, reading in extinction coefficients. Accompanying tables give the corresponding per cent transmittancy. The eight filters and a number of attachments provide for many optical measurements.

A third example is the neutral wedge photometer devised by Clifford (*3*). A calibration curve is constructed for a given determination by noting the position of the movable wedge for a series of known solutions.

Photoelectric Type. Probably the most notable changes in colorimetric instruments used by analysts followed the introduction of photoelectric cells. Both barrier-layer and photoemission types of cells are employed. Their use in filter photometers led the National Bureau of Standards to issue an extensive circular (*12*) which presents a critical evaluation of such devices.

Many variations are found in the electrical and optical details of the schemes which have been suggested. A few of these differences are noted in connection with specific examples cited below. While the general tendency has been to use arbitrary scales, a few instruments are designed to read transmittancy or amount of desired constituent directly. They are usually classified on the basis of the number of photocells used.

One-Cell Instruments. When only one photocell is used, the essential parts of the arrangement consist of a light source, a container for the sample, a photocell to receive the transmitted light, and some means of measuring the response of the photocell. In Figure 4 both the general appearance and the schematic arrangement of parts are shown for Sheard and Sanford's design (*39*), one of the earlier instruments. Similar devices are those of Evelyn (*8*), the Fisher Scientific Co. (*10*), Müller (*34*), and Yoe and Crumpler (*54*).

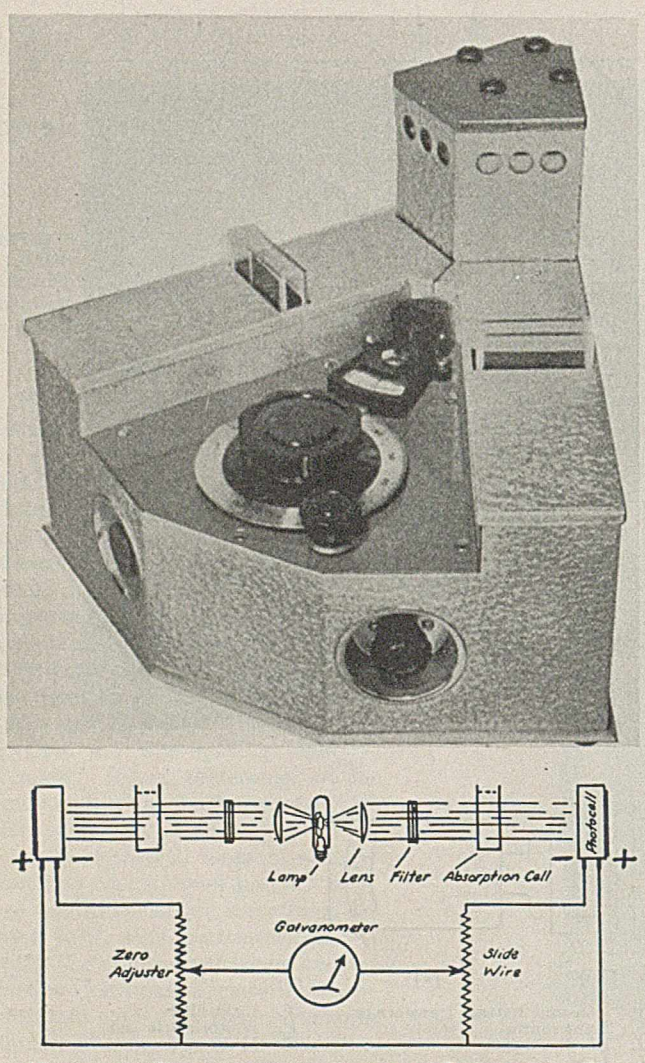


FIGURE 5. PHOTOMETER OF AMERICAN INSTRUMENT CO.

In order to ensure constancy in the illuminant, and consequently in the response of the photocell, the electric current for the illuminant is provided by a storage battery or a constant power transformer. The response of the photocell is detected by means of a sensitive galvanometer or a microammeter. While it is generally necessary to calibrate the instrument for a given determination by correlating concentration of the desired constituent with microammeter or galvanometer readings, a direct reading scale may be incorporated. In Kudor's design (*26*) a different direct-reading scale can be turned into place for each of a number of constituents to be determined.

Evelyn's arrangement provides for the use of test tubes for the solution instead of the usual optically plane cells. It may also be used for samples of a micro size.

The absorption cell is used in different ways. If an arbitrary calibration curve is constructed, only one cell is needed and it may have any usable dimensions as long as it is always used in the same way for both known and unknown solutions. When the transmittancy is desired, preferably the cells should have optically plane faces and a definite thickness. Either the solvent and solution are measured successively in the same cell, or two optically interchangeable cells may be employed, one for the solution and one for the solvent.

Two-Cell Instruments. In order to avoid the provisions necessary to ensure constancy of operating current for the light source in one-cell instruments, investigators rather early proposed two-cell arrangements based on the idea that fluctuations would affect the two cells equally and thus be compensated.

One type of arrangement for two photocells is illustrated in Figure 5. The essential differences from one-cell arrangements are that two beams of light come from the illuminant, one going to each photocell, and that the response meter, in this case a galvanometer, is used as a null-point instrument. The ordinary alternating current line furnishes current for the illuminant.

The instruments described by Exton (*9*) and by Withrow, Shrewsbury, and Kraybill (*51*) are examples of the use of two photoemission cells. The latter paper stresses particularly the electrical problems involved.

The use of barrier-layer cells has been more common, the apparatus of the American Instrument Co. (*2*), Keane and Brice (*24*), Lange (*36*), and Spekker (Hilger) (*42*) being representative examples of those currently advertised. The author has had good results with one adapted from the design of Wilcox (*50*) and equipped with Aklo filters to diminish the response lag in the photronic cells by absorbing the infrared radiation from the illuminant. In such instruments the two photocells may be used either in a series-opposing or a parallel connection. Brice (*5*) has reviewed various proposals for the circuits and given a critical analysis of the arrangements and performance of one modification.

Certain modifications of this type of instrument make it possible to measure transmittancy directly by using two optically interchangeable absorption cells simultaneously, one containing the solvent only in one beam and one containing the solution in the other beam, as shown in Figure 5. Also, the absorption cell may be used in the same way as in one-cell instruments.

SPECTROPHOTOMETERS. Since the general role of spectrophotometry in colorimetry was presented in an earlier paper (*30*), reference should be made to it for information on visual and photoelectric types of instruments and their general analytical applications. The material presented here is limited to what seems necessary to relate spectrophotometers to the types of colorimetric instruments already discussed.

On account of their cost and the knowledge and experience required to keep them operating reliably, spectrophotometers are undoubtedly to be considered still as instruments primarily for research and standardization. They constitute, however, in the opinion of physicists, the most fundamental method of colorimetry available.

One uses a rather wide spectral band from the illuminant in filter photometers, the particular region depending upon the filter selected. In contrast to this, spectrophotometers have a single or double monochromator in which prismatic dispersion of light from the illuminant enables one to select any wave length desired. In addition, adjustment of the slits regulates the width of spectral band entering the photome-

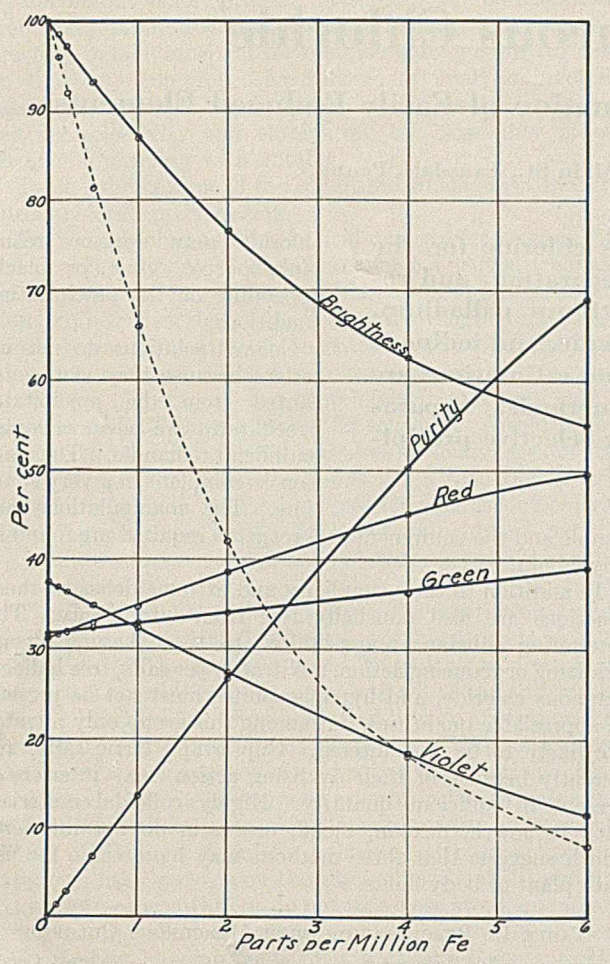


FIGURE 6. COLORIMETRIC VALUES FOR SOLUTIONS CONTAINING DIFFERENT AMOUNTS OF IRON

Broken curve is percentage transmittancy

ter. Reference to Figure 2 shows the difference in the data obtained on a filter photometer and on a recording spectrophotometer set for a 5 mμ spectral band. Hogness, Zscheile, and Sidwell (*21*) used their photoelectric instrument with a band width of less than 3 Å. in the visible region.

The work of Mehlig (*28*) may be cited as representing colorimetric determinations made by means of a spectrophotometer. Whereas one usually thinks of colorimetric methods as applicable only to relatively small concentrations of material, this work included determinations in ores of as much as 21 per cent of copper and 57 per cent of iron.

Spectrophotometric curves provide the fundamental data for the calculation of numerical specifications of color for definite illumination and for an assumed normal observer. The data are expressed in tristimulus values as percentages of red, green, and violet, or in monochromatic terms as dominant wave length, in millimicrons, and as percentages of relative brilliance and colorimetric purity. Thus far analysts have made little use of such data. De Almeida (*1*) suggested determining pH values from a curve coordinating dominant wave length and pH for a given indicator. Using a recording spectrophotometer (*33*), Hardy's ten selected ordinates for calculation (*19*), and a recently described calculator (*45*), colorimetric specifications may be obtained fairly rapidly. Figure 6 illustrates how they may be correlated with concentration. The data shown are for solutions of iron treated with *o*-phenanthroline and were calculated for illuminant *C* from curves published recently (*11*). It is apparent that a relatively high sensitivity may be obtained for this system by using the curves for relative brilliance or colorimetric purity. With less sensitivity any one of the tristimulus values could be used, green being the least sensitive of the three. It would seem possible, therefore, to use a stimulimeter in this way. The dominant wave length curve is too nearly horizontal to be of value. None of these curves gives the sensitivity of the transmittancy curve itself, which is shown as a broken line for the values at 506 mμ, the peak of the absorption band. Furthermore, the transmittancy does not necessitate any calculations.

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The Use of Mercurous Chloride

For the Separation, Detection, and Estimation of Easily Reduced Elements

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The use of mercurous chloride for the detection, estimation, separation, and recovery of gold, silver, platinum, palladium, selenium, tellurium, arsenic, and iodine is described. Detections and estimations are made by colorimetric methods. Separations are made by using selective precipitating conditions.

A GOOD many years ago during an attempt to prepare arsenic cyanide, it was observed that mercurous chloride would precipitate arsenic and a number of other easily reduced elements. Later these reactions were investigated in more detail and new methods were described (*2*, *3*), adapting the reducing action of mercurous chloride specifically to the detection, estimation, and separation of minute quantities of gold, platinum, palladium, selenium, tellurium, and arsenic.

Although these reactions are simple and easy to observe, the previous literature on the subject had apparently been confined to an occasional statement in textbooks that soluble mercurous salts precipitate gold from solution. However, in 1927 Yasuda (*4*) reported that magnesium added to a strong acid solution of mercuric chloride would precipitate a cloud of finely divided metallic mercury, which would carry down as an amalgam any gold or silver in the solution. The precipitated amalgam was then fire-assayed to determine gold and silver. No significance was attached to the fact that the reduction of mercuric chloride with magnesium first produced a cloud of mercurous chloride, which in all probability was never completely reduced to metallic mercury. Yasuda used his method to determine the gold content of sea water near Japan and reported quantities of from $^1/_3$ to 1 part per 100 million.

In 1935 Caldwell and McLeod (*1*) confirmed the effectiveness of Yasuda's procedure and among other things stated that as little as one part of gold in four billion parts of water could be quantitatively recovered. They called the precipitating agent a cloud of mercury mercurous chloride.

General Characteristics of the Reaction

When mercurous salts are added to suitable solutions of gold, platinum, palladium, selenium, tellurium, and arsenic, these are reduced and precipitated as elements, the mercurous salts being oxidized to the mercuric. A general equation for the reaction is

$$HgX + MX = HgX_2 + M$$

This reaction is unidirectional under favorable conditions and proceeds rapidly even with highly insoluble mercurous salts such as the halides. These very insoluble mercurous halides not only rapidly throw the elements out of solution but quickly and completely adsorb them. These precipitated elements are colored, depending both upon the particular element and the amount present, and these colors afford the means for detection and estimation.

Powdered mercurous chloride is the most effective precipitating agent. It is highly insoluble and very reactive, and being heavy settles rapidly, carrying down the reduced and adsorbed elements. This makes separations easy. Mercurous chloride is also opaque and white and offers a good background for observing the colors of the adsorbed elements—for example, a very small amount of gold on mercurous chloride may appear cream, pink, purple, gray, or black, depending on the precipitating conditions.

Colored solutions do not interfere because they can be decanted from the precipitate. Precipitates are never colloidal or difficult to handle. The reaction is complete in a very short time. The manipulations are simple and the equipment and reagents required are found in any laboratory.

In addition to their simplicity and extreme delicacy, these reactions are also unusually free from interference. The interfering substances are limited to those having strong oxidizing or reducing action. Nitrates, per salts, free halides, stannous chloride, and hypophosphites must not be present in appreciable quantities, but among this group only nitrates are likely to be encountered. Cupric and ferric salts, apparently because of their oxidizing action, may interfere if present in sufficient quantity. Highly colloidal materials such as starch, dextrin, blood, or casein do not interfere, which suggests that these methods may have value for use with plant or body fluids.

TABLE I. PRECIPITATION WITH MERCUROUS CHLORIDE

Element Present	HCl Required for Precipitation in Cold	Boiling HCl Solution, 0.02% $HgCl_2$	Preferred Conditions for Precipitations
Au	0 to concd.	0 to concd.	About 2% HCl, warm
Pt	0 to slight	0 to concd.	About 2% HCl, 0.02% $HgCl_2$, hot
Pd	0 to concd.	0 to concd.	About 2% HCl, warm
Se	6 to 15%, partial 16 to concd., complete	6 to 15%, partial 16 to concd., complete	About 20% HCl, cold
Te	6 to 15%, partial 16 to concd., complete	6 to 15%, partial 16 to concd., complete	About 20% HCl, cold
As (ous)	27% to concd.	27% to concd.	About 30% HCl, cold
I	0 to slight	None	Very slightly acid, cold

Silver is precipitated quantitatively by mercurous chloride but this reaction has not been investigated in detail. Substantial quantities of silver on mercurous chloride produce a cream color but small quantities are difficult or impossible to detect. The metals ruthenium, rhodium, iridium, and osmium usually occurring with platinum and palladium are not precipitated by mercurous chloride.

This study of the reducing action of mercurous chloride has been principally confined to very dilute solutions of easily reduced elements.

Specific Characteristics of the Reaction

Mercurous chloride may or may not react with solutions of these easily reduced elements, depending upon conditions of acidity and temperature or the presence of interfering chemicals, and among these conditions a number have been found which are selective or specific with regard to one or more elements. This has permitted working out a scheme of separations as well as the determination of one element in the presence of others. Among the elements precipitated

by mercurous chloride, gold, platinum, and palladium are frequently found together as are selenium, tellurium, and arsenic; in fact, all six of these elements may occur together, so that any comprehensive scheme of analysis involving mercurous chloride required that conditions be found permitting selectivity and eliminating the possibility of one element interfering with another.

Table I shows some of the conditions which affect the mercurous chloride precipitation.

The second column shows the acid required for precipitation from cold solutions. Gold comes down in a neutral or concentrated acid solution. Platinum comes down in a neutral solution, but not when the acidity is more than slight. Palladium comes down in a neutral or concentrated acid solution. Selenium does not come down at all under 6 per cent, incompletely from 6 to 15 per cent, but completely from 16 per cent to concentrated. Tellurium reacts like selenium. Arsenic, which must be in the trivalent form, requires an acidity greater than 27 per cent. The iodine reaction is not a reduction as with the other elements, but a transposition, mercurous chloride becoming mercurous iodide and therefore highly colored. In very dilute solutions iodides do not transpose when the acidity is more than slight.

The third column of Table I shows the reactions in boiling acid solutions to which 0.02 per cent of mercuric chloride has been added. The mercuric chloride is added to prevent the mercurous chloride from decomposing with heat and turning gray or black which, of course, would mask any other color coming from the precipitated elements. Gold comes down from neutral or concentrated acid as in a cold solution. Platinum comes down with any acid concentration, whereas in a cold solution there is no reaction with acidity greater than slight. Palladium, selenium, tellurium, and arsenic precipitate with heat very much the same as without. Iodides in very dilute concentrations do not transpose at all, apparently owing to the effect of mercuric chloride rather than heat.

By simply changing the acidity, a variety of separations is possible. With a neutral solution the first three elements come down while the others do not. With cold 16 per cent acid only arsenic, platinum, and iodides remain in solution; with 30 per cent acid only platinum and iodides remain in solution; with boiling the platinum is precipitated, etc.

Table II. Outline of Separations

	2% HCl solution of Au, Pd, Pt, Se, Te, and As Add 1% oxalic acid, boil to precipitate Au, filter
Au precipitate	Solution of Pd, Te, Se, Te, and As Cool, add HgCl to precipitate Pd, filter
Pd, HgCl precipitate	Solution of Pt, Se, Te, and As Add 0.02% $HgCl_2$, add HgCl, boil, precipitate Pt, filter
Pt, HgCl precipitate	Solution of Se, Te, and As Add HCl to 20%, cool, add 5% $NaHSO_3$, stand, boil, precipitate Se, filter
Se precipitate	Solution of Te and As Cool, add HgCl, precipitate Te, filter
Te, HgCl precipitate	Solution of As Add HCl to 28%. Add HgCl, precipitate As

In the last column the precipitating conditions usually employed for separations or estimations are given. For gold, a 2 per cent acid solution is used. Gold comes down completely and rapidly in the cold but the color is usually gray, resembling platinum or palladium. On warming the precipitate on a water bath for a minute or two a characteristic purple or pink will develop.

For platinum a 2 per cent acid solution is used to which 0.02 per cent of mercuric chloride has been added. After adding the mercurous chloride, the mixture is heated on a boiling water bath for about 15 minutes, followed by a gentle boiling for about one minute which is usually sufficient for complete precipitation of platinum.

Palladium will precipitate readily in the cold when a 2 per cent acid solution is used, but some heat will intensify the coloring slightly.

With both selenium and tellurium a 20 per cent hydrochloric acid solution is used, without heat. The colors from these elements fade with heat or on standing several hours at room temperature. A 30 per cent acid solution is suitable for arsenic, without heat. If arsenic is in the (ic) form it should be reduced, sulfurous acid or sulfites being suitable. The color from arsenic also fades slowly with heat or long standing at room temperature. The iodide transposition will take place when the solution is slightly acid to litmus and cold.

Table II shows an outline of some separations made with the help of mercurous chloride.

Starting with a 2 per cent hydrochloric acid solution of gold, palladium, platinum, selenium, tellurium, and arsenic, add 1 per cent oxalic acid, boil to precipitate gold, and filter. Dissolve the precipitated gold with chlorinated acid, boil to remove chlorine, and precipitate with mercurous chloride for identification or estimation. The solution, which now contains palladium, platinum, selenium, tellurium, and arsenic, should be cooled to room temperature and palladium precipitated with mercurous chloride. Filter. The precipitate is a mixture of mercurous chloride and palladium, and the solution contains platinum, selenium, tellurium, and arsenic. Add 0.02 per cent of mercuric chloride followed by mercurous chloride and boil to precipitate platinum. The precipitate is a mixture of mercurous chloride and platinum.

If desired, mixtures of mercurous chloride with palladium, gold, or platinum can be separated by subliming the mercurous chloride. Make up the solution, which now contains selenium, tellurium, and arsenic, to 20 per cent hydrochloric acid and cool. Add 5 per cent of sodium acid sulfite, let stand for 15 minutes, boil to precipitate selenium, and filter. The precipitate is selenium alone which can be dissolved with chlorinated acid and reprecipitated with mercurous chloride. Cool the solution containing tellurium and arsenic, precipitate the tellurium with mercurous chloride, and filter. The precipitate is a mixture of tellurium and mercurous chloride and the solution contains arsenic which is precipitated with mercurous chloride after the acidity is brought up to 28 per cent.

General Method for Detections and Estimations

Detections are based on various colors, and estimations are based on the shades of such colors produced by the precipitated element. The shades produced by one element on a fixed amount of mercurous chloride depend principally upon the amount precipitated but also to a minor extent upon the solution concentration of the element before precipitation. Color variations may also be caused by temperature and solution purity; however, these possible sources of error are reduced to a minimum when standard controls are carried along under the same conditions as the test sample. It is usually desirable to extract a portion of the test sample with mercurous chloride and use this portion as a base for preparing a standard control. For each element there is a range over which the variation of color per unit of element is maximum. On either side of this sensitive range little change in color is produced by increased or decreased adsorption.

Hydrochloric acid is used to control the acidity of the solution; it should first be boiled with about 1 per cent of mercurous chloride, then permitted to stand for 48 hours, when the clear acid can be decanted for use. Sulfuric, hydrochloric, and possibly other nonoxidizing acids can be used.

In general the procedure for estimating has been to put 1 ml. or less of the test solution in a 100-ml. beaker, dilute to 5 ml. using hydrochloric acid or water as required, and then add 0.1 gram of mercurous chloride. With a little shaking the reaction is complete in a few minutes, after which the mercurous chloride is collected for observation by tilting the beaker.

Table III shows the colors developed on mercurous chloride by adsorption of the elements listed when 0.1 gram of mer-

TABLE III. COLORS ON MERCUROUS CHLORIDE

(Using 0.1 gram of HgCl and 5 ml. of solution)

Element	0.1 Mg.[a]	0.01 Mg.[a]	0.001 Mg.[a]	0.0001 Mg.[a]
Au	Purple	Purplish pink	Light pink	Faint pink
Pt	Dark gray	Light gray	Cream	Light cream
Pd	Gray-black	Light gray	Grayish cream	Light cream
Se	Salmon	Pinkish cream	Light cream	...
Te	Brownish yellow	Brownish cream	Light cream	...
As	Brown	Pinkish brown	Pink	Cream
I	Greenish yellow	Light yellow	...	...

[a] Element present in 5 ml. of test solution.

TABLE IV. ACCURACY OF METHOD

Element	Accuracy with Approximately 0.1 Mg. of Element	Minimum Visible on 0.1 Gram of HgCl
	%	*Mg.*
Au	± 3	0.00005
Pt	± 5	0.0001
Pd	± 3	0.00005
Se	± 5	0.0002
Te	±10	0.0005
As	± 5	0.000005
I	± 5	0.003

curous chloride is used with 5 ml. of test solution. Gold shades off from purple to pink, platinum from dark gray to cream, palladium from gray-black to cream, selenium from salmon to cream, tellurium from brownish cream to cream, arsenic from brown to pink to cream, and iodide from greenish yellow to light yellow. In each case the maximum color change is from about 0.1 to 0.01 mg. and whenever possible a solution carrying about 0.1 mg. should be used for estimations. With the larger amount the percentage of error is less.

Table IV shows the per cent accuracy of the method when working with about 0.1 mg. of element. The figures are an average of tests made with reasonably pure solutions and seem to compare favorably with other colorimetric estimations. The third column shows the smallest amount of each element on 0.1 gram of mercurous chloride visible to the unaided eye.

Arsenic Tests

A number of basically different chemical methods have been developed for detecting and estimating minute quantities of arsenic. The March and Gutzeit methods reduce arsenic to arsene, but require special apparatus and an experienced operator to give the best results. A strychnine or cocaine molybdate reagent has been proposed which gives a measurable turbidity with arsenic. Methods employing stannous chloride, sodium hypophosphite, or acid sodium thiosulfate have been used extensively to reduce solutions of arsenic, giving a brown color suitable for colorimetric estimations. These last methods require comparatively pure and colorless test solutions, and are somewhat lacking in accuracy and sensitivity. A method based on the formation of molybdenum blue from arsenomolybdate is probably among the most accurate and suitable for determinations over a rather broad range of arsenic content. Test solutions, however, should be colorless and a number of elements interfere. Finally, a novel test has recently been described, depending on the odor given off by certain molds feeding on an arsenic solution.

Considering the possibilities of those methods just mentioned, it seems that the mercurous chloride method has certain outstanding advantages. Within a limited range its accuracy is equal to the best. The sensitivity apparently exceeds all others by a substantial margin. No apparatus is required and the manipulations are very simple. Interference is reduced to a negligible factor for many solutions; colored solutions do not interfere.

Additional Applications

The use of mercurous chloride offers one of the most delicate colorimetric chemical tests known and its use for this purpose has been emphasized; however, mercurous chloride containing adsorbed gold, silver, platinum, or palladium may be fire-assayed without difficulty by methods such as those described by Caldwell and McLeod (*1*).

Mercurous chloride is also highly effective and economical in recovering gold, silver, platinum, and palladium from extremely dilute solutions. Commonly used precipitating agents do not offer simultaneous adsorption and for that reason precipitate these metals from dilute solutions as an extremely fine suspension, frequently colloidal and accordingly very difficult or impossible to recover.

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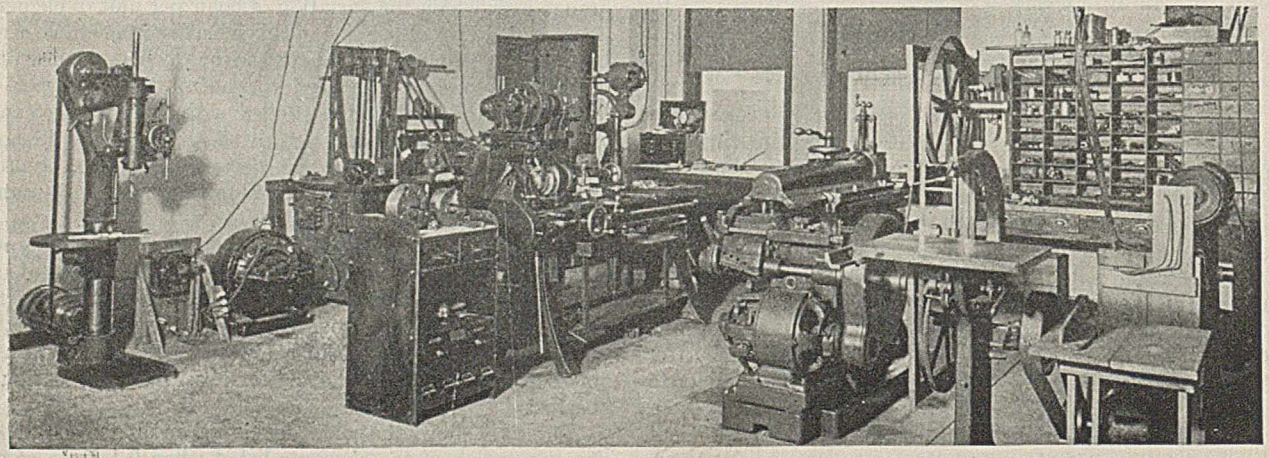

Courtesy, U. S. Bureau of Mines

Determination of Halogens in Organic Compounds

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SINCE 1923 when Fajans and Hassel (*6*) applied adsorption indicators to the argentometric determination of inorganic halides, the use of these indicators for this purpose has become rather extensive (*9, 10*). In the field of organic analysis, however, they have been used successfully only for semimicroanalyses. Bobranski and Sucharda (*2, 3*) and later Hardy (*7*) using a 2- to 3-centigram sample for analysis burned it in an atmosphere of oxygen over a platinum catalyst. In the case of chlorides and bromides the combustion gases were passed over barium carbonate and the resulting barium halide was titrated with silver nitrate using eosin or fluorescein as adsorption indicator. For iodides, a 40 per cent solution of sodium sulfite was used as the absorption liquid, and the iodide formed after removal of excess sulfite by barium carbonate was titrated with silver nitrate using eosin as an indicator. Holscher (*8*) burned the sample in an air stream and passed the combustion gases through a 5 per cent hydrogen peroxide solution. After buffering with sodium acetate, he titrated the halide formed using dichlorofluorescein or eosin as adsorption indicator.

On a macroscale Bambach and Rider (*1*) found that dichlorofluorescein could be used successfully in the case of inorganic halides and organic hydrochlorides in alcohol-water solutions. They suggested the possibility of applying this titration in conjunction with the Stepanow method of reduction to the determination of the halogen content of organic substances.

In the work reported in this paper it was found that a combination of a modified Stepanow procedure and a subsequent titration with silver nitrate using adsorption indicators was a reliable and accurate method for determining the halogen content of a variety of organic halides.

Experimental Procedure

The procedure employed for the reduction of the organic halides was based upon the modification of the Stepanow method proposed by Cook and Cook (*4*). The amounts of sodium and alcohol employed were calculated from the empirical rules of Drogin and Rosanoff (*5*), except for halogenated nitro compounds where the 150 per cent increase recommended by Cook and Cook was used.

A weighed sample of thoroughly desiccated organic halide (equivalent to approximately 25 cc. of 0.1 *N* silver nitrate, except in cases of halogenated nitro compounds where an amount approximately equivalent to 10 cc. of silver nitrate was used in order to avoid too great dilution of the solution to be titrated) was placed in a 250-cc. Erlenmeyer flask fitted with a reflux condenser, the constricted tip of which had been removed. In the case of solids the samples were weighed into small vials which were dropped into the Erlenmeyer flask. For the analysis of liquids, small vials were made by heating 6-mm. glass tubing until one end was sealed and pressing this end against a flat surface while soft. This tubing was then cut off at a length of 3 cm., resulting in a small vial which stood erect on the balance pan. During weighings the open end of the tube was closed by a small cork stopper. In transferring to the Erlenmeyer flask the cork was removed and the vial plus the liquid was dropped into the alcohol which was placed in the flask prior to the addition of the sample.

The reductions proceeded just as effectively in the Erlenmeyer flask as in the Kjeldahl flask recommended by Cook and Cook, while the titrations in the former were carried out much more easily than in the latter where the long neck interfered with the fall of drops from the buret to the flask.

The required amount of absolute alcohol previously distilled over metallic sodium in order to remove aldehydes was then added and the flask warmed over a low Bunsen flame until the sample had dissolved. Absolute alcohol free from aldehyde was found necessary for the success of the determination since otherwise, after reduction, the liquid was found to be colored so darkly, owing to polymerization of the aldehyde in the alkaline medium, that the final titration could not be performed. The burner was then removed and the required amount of sodium (Baker's C. P. grade) cut into rods about 2.5 cm. long was introduced through the top of the condenser. At least 0.5 hour was allowed for the dissolution of the sodium and at no time were there more than three pieces of sodium in the flask. During the latter part of the addition, the reaction of the sodium was aided by a small flame under the flask. The solution was then gently refluxed for one hour, after which it was allowed to cool and diluted with about 15 cc. of water, at first drop by drop and then by larger amounts as the violence of the reaction subsided. The flask was now held under running water, and two drops of phenolphthalein were added, followed by addition of approximately 6 *N* nitric acid drop by drop until the solution was decolorized. The required amount of adsorption indicator was next added (8 drops of dichlorofluorescein in the case of chlorides and 2 drops of eosin for bromides and iodides) and the solution was titrated with standard 0.1 *N* silver nitrate until the color changes described below occurred.

As the silver nitrate was added the silver halide first precipitated in colloidal form. As more reagent was added the precipitate coagulated and settled to the bottom of the flask in the form of flocs. Just before reaching the end point the flocs formed a large number of grainy particles which in the case of dichlorofluorescein became distinctly pink and in the case of eosin changed from a pale pink coloration to a bright rose red at the equivalence point. These color changes were best observed by keeping the contents of the flask in motion during the titration.

Experimental Results

The results of analyses of a variety of halogenated organic compounds using the above described procedure appear in Table I.

TABLE I HALOGEN CONTENT OF COMPOUNDS ANALYZED

Compound	Determinations	Theoretical Halogen	Halogen Found	Average Deviation
		%	%	*P. p. m.*
Chloroacetamide	5	37.91	38.06	4.7
Chlorobenzene	5	31.52	31.49	5.1
p-Dichlorobenzene	5	48.26	48.35	2.7
m-Nitrochlorobenzene	3	22.52	22.20	3.2
Hexachlorobenzene[a]	4	74.74	74.71	0.3
Hexachloroethane	4	89.84	89.45	1.7
Bromobenzene	5	50.92	50.81	0.8
p-Dibromobenzene	5	67.77	67.67	1.6
m-Nitrobromobenzene	4	39.57	39.60	2.5
p-Nitrobromobenzene	4	39.57	39.63	1.5
1,3,5-Tribromobenzene	4	76.19	76.13	1.6
2,4,6-Tribromoaniline	4	72.71	72.48	1.3
Dibromocinnamic acid	5	51.88	51.99	2.3
3-Bromocamphor	5	34.59	34.69	3.2
Iodoform	5	96.70	96.50	2.4

[a] The reduction of hexachlorobenzene because of difficult solubility required quantities of alcohol and sodium 25% in excess of that calculated from the empirical rules.

Dichlorofluorescein was used as the indicator for chlorine determinations and eosin for bromine and iodine. Attempts to use dichlorofluorescein for bromine determinations led to unsatisfactory results. The precipitate darkened so quickly that the detection of the equivalence point during the titration became very difficult or impossible. These results differ from those secured by other investigators (*8*) in the determination of the halogen content of inorganic halides where it was found that dichlorofluorescein could be used for all three.

In the case of the iodine determination it was found that 6 *N* nitric acid could be employed for the acidification prior to titration instead of the very dilute acid used by earlier investigators. The addition was made very slowly while the flask was cooled in a bath of ice and water. Five determinations of the iodine content of iodoform showed no appreciable oxidation of the iodide.

Summary

The halide content of a variety of halogenated organic compounds can be determined satisfactorily by a modified Stepanow method of reduction, followed by titration with silver nitrate using dichlorofluorescein for chlorine and eosin for bromine and iodine as adsorption indicators.

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Calcium Oxalate Monohydrate as a Weighing Form for Calcium

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PROBABLY the most accurate weighing form for calcium after precipitation as the oxalate is the carbonate (*8*), but the close control of temperature required in the method of Willard and Boldyreff makes the method unavailable to analysts who do not have the necessary equipment. Although good results can be obtained by weighing calcium as the oxide, special precautions must be taken to prevent the absorption of moisture, and the low molecular weight is a disadvantage. Calcium sulfate is not a convenient weighing form and is used only infrequently.

Calcium oxalate monohydrate would possess distinct advantages as a weighing form if the accuracy were sufficiently high. The possible sources of error involved include retention of foreign water (adsorbed and/or included) after drying, loss of hydrate water on drying, and coprecipitation of ammonium salts or oxalic acid. The ideal procedure from the standpoint of simplicity would involve drying the washed precipitate at room temperature after treatment with alcohol and ether or with acetone. Goy (*3*), who first proposed calcium oxalate monohydrate as a weighing form, dried the precipitate at 100° to 105° C. Dick (*1*), on the other hand, washed the precipitate with alcohol and ether, placed the crucible for a short time in a vacuum desiccator, and then weighed. Others (*7*) have used the same or a similar procedure. Moser and von Zombory (*5*) raised objections to the method of Dick, and according to them the results obtained by this procedure are much too high (1.6 to 3 per cent). Haslam (*4*) also obtained high results by Dick's method and by drying at 100° C. The monohydrate does not appear to be in good repute as a weighing form for calcium in macroanalysis, at least not in America, but it is frequently used in microanalysis. Since the statements in the literature regarding the use of the monohydrate as a weighing form are contradictory, the authors have precipitated calcium oxalate under various conditions and tested the suitability of this method of weighing calcium.

Experimental

In most of the determinations the amount of calcium taken was obtained from the weight of calcium carbonate of known calcium content. Two preparations of calcium carbonate were used:

PRODUCT I. This product was prepared by adding 0.05 *M* calcium chloride solution to excess hot 0.05 *M* ammonium carbonate solution, washing the precipitate with hot water, and drying at 150° C. The preparation contained only a trace of chloride and magnesium was not detectable. The calcium carbonate content of the product was determined acidimetrically by adding a slight excess of hydrochloric acid and back-titrating with sodium hydroxide. The hydrochloric acid was standardized gravimetrically by determining the chloride as silver chloride. Weight burets were used throughout. Three determinations yielded the values 99.885, 99.87, and 99.84 per cent of calcium carbonate or an average of 99.87 per cent. Known amounts of this product were weighed out, dissolved in hydrochloric acid, and used in most of the determinations of Table I. In a few cases a calcium chloride solution was used which had been standardized by evaporating a suitable volume to dryness and converting the residue to calcium sulfate.

PRODUCT II. This product, which had been prepared by J. J. Lingane of this laboratory for use in the J. Lawrence Smith method of decomposition for the alkali determination, was obtained by dissolving C. P. calcium carbonate in hydrochloric acid, precipitating with ammonia and ammonium carbonate in hot solution, and drying the washed precipitate at 130° C. Gravimetric determination of calcium in the product (calcium carbonate as weighing form), with a correction for solubility loss of of calcium oxalate, yielded the value 98.87 per cent of calcium carbonate. The calcium carbonate content was also determined acidimetrically by using hydrochloric acid which had been standardized against potassium bicarbonate specially prepared as a primary standard. Three titrations with weight burets gave 98.89, 98.91, and 98.91 per cent of calcium carbonate. The value 98.90 per cent has been used in calculating the amount of calcium from the weight of calcium carbonate taken. Product II was used in all the determinations of Table II.

The precipitations were made as indicated in the tables. Porous porcelain and sintered-glass crucibles were used to collect the precipitates, and 50 to 75 ml. of cold water were used for washing.

Tables I and II give some of the results obtained in investigating the suitability of calcium oxalate monohydrate as a weighing form. A number of the results in Table I were obtained in an earlier study (*6*) of the water content of calcium oxalate in which precipitation was made in neutral,

TABLE I. $CaC_2O_4.H_2O$ AS A WEIGHING FORM FOR CALCIUM

(Precipitated by ordinary methods)

No.	Conditions of Precipitation	$CaC_2O_4.H_2O$ Taken	Temperature of Drying	Time of Drying	$CaC_2O_4.H_2O$ Found	Error	
		Gram	*° C.*	*Hours*	*Gram*	*Mg.*	*%*
1	50 ml. of 2.0% $(NH_4)_2C_2O_4.H_2O$ added slowly to 200 ml. of hot neutral calcium chloride solution. Filtered after cooling to room temperature	0.4458	25 (R. H.[a] = 57%)	120	0.4495	+3.7	+0.83
			100–105	5	0.4470	+1.2	+0.27
			100–105	15	0.4462	+0.4	+0.09
			100–105	15	0.4463	+0.5	+0.11
			105–110	46	0.4459	+0.1	+0.02
			110–115	18	0.4451	−0.7	−0.16
			115–120	22	0.4433	−2.5	−0.56
			115–120	20	0.412	−34	−7.6
2	50 ml. of 2.0% $(NH_4)_2C_2O_4.H_2O$ added slowly to 200 ml. of hot calcium solution containing 1 drop of 2 *N* ammonium hydroxide and 2 grams of NH_4Cl	0.3758	105	23	0.3782	+2.4	+0.64
			105	25	0.3780	+2.2	+0.59
			110	69	0.3790	+3.2	+0.85
			115–120	45	0.3665	−9.3	−2.5
3	As in (2) except 20 ml. of 2.5 *N* ammonia and 2 grams of NH_4Cl present	0.4518	115–120	5	0.4363	15.5	−3.4
			115–120	2	0.4346	17.2	−3.8
			115–120	3	0.4320	19.8	−4.4
			110	15	0.4112	40.6	−9.0
4	50 ml. of 2.0% $H_2C_2O_4.2H_2O$ added to 200 ml. of hot calcium solution containing 1 ml. of concentrated hydrochloric acid; solution then neutralized with 1 *N* ammonia and 0.5 ml. added in excess. Filtered when cold	0.4501	115–120	1	0.4523	+2.2	+0.49
			115–120	2	0.4516	+1.5	+0.33
			115–120	17	0.4519	+1.8	+0.40
5	As in (4) except 50 ml. of 2.0% $(NH_4)_2C_2O_4.H_2O$ used	0.3641	25	24	0.3678	+3.7	+1.01
			25 (R. H. = 57%)	24	0.3680	+3.9	+1.07
			25 (R. H. = 0%)	6 weeks	0.3672	+3.1	+0.85
			105	1	0.3673	+3.2	+0.87
			115	2	0.3672	+3.1	+0.85
6	50 ml. of 2.0% $(NH_4)_2C_2O_4.H_2O$ added to 175 ml. of hot calcium solution containing 2 ml. of concentrated hydrochloric acid; neutralized with 6 *N* ammonia added rapidly dropwise. Filtered after two days	0.7221	25	...	0.7249	+2.8	+0.39
			107	2	0.7206	−1.5	−0.21
			110	2	0.7195	−2.6	−0.36
			25	1	0.7225	+0.4	+0.06
			105	18	0.7217	−0.4	−0.06
			105	2	0.7213	−0.8	−0.11
7	As in (6) except 1 ml. of concentrated hydrochloric acid. Filtered after 4 hours	0.7219	25	...	0.7273	+5.4	+0.75
			105	5	0.7219	0.0	0.00
			105	1	0.7221	+0.2	+0.03
			105	40	0.7222	+0.3	+0.04
8	25 ml. of 4% $(NH_4)_2C_2O_4.H_2O$ added to 100 ml. of cold calcium solution containing 1 ml. of 4 *N* acetic acid. Digested at 90° for 20 hours, cooled, filtered, and precipitate finally washed with alcohol and ether	0.3648	25	...	0.3652	+0.4	+0.11
			105	20	0.3649	+0.1	+0.03
			25 (R. H. = 29%)	24	0.3650	+0.2	+0.05
9	As in (8)	0.3648	25	...	0.3657	+0.9	+0.25
			105	18	0.3654	+0.6	+0.16
			110	0.5	0.3653	+0.5	+0.14
			25 (R. H. = 29%)	24	0.3656	+0.8	+0.22

[a] Relative humidity.

acid, and ammoniacal medium. Table II contains the results obtained by applying the precipitation method of Willard and Chan (*9*) in which the strongly acid solution of calcium containing an excess of oxalate is heated with urea; the ammonia produced by the hydrolysis of the urea slowly neutralizes the acid and a very coarse precipitate of calcium oxalate is thus formed. In the column headed "temperature of drying," 25 indicates that the precipitate was dried at room temperature by drawing air through the crucible for 5 to 10 minutes after washing with alcohol and ether (Table I) or acetone (Table II). In some cases the precipitate was dried further over concentrated sulfuric acid and this is indicated by R. H. (relative humidity) = 0 per cent. In other cases the precipitate was placed in a hygrostat of saturated sodium bromide, calcium chloride, or potassium chloride solution, respectively, and the corresponding relative humidity is then indicated. The same precipitate was used throughout each numbered experiment and was dried under various conditions and time in the order shown. In some of the experiments of Table II the precipitate was finally ignited to calcium carbonate according to the directions of Willard and Boldyreff (*8*), and in a few cases the precipitate was titrated with potassium permanganate (using weight burets) by the method of Fowler and Bright (*2*), the permanganate being standardized against Bureau of Standards sodium oxalate dried at 105° C. The permanganate titrations were made by Donald Smith.

Discussion of Results

Calcium oxalate monohydrate precipitated in the usual way (Table I, Nos. 1 to 7) from neutral, ammoniacal, or acid medium retains foreign water after air-drying at room temperature, the amount ranging from about 0.3 to 1 per cent or more. The customary method of precipitation from hydrochloric acid solution, with final neutralization by ammonium hydroxide, gives decidedly high results (variable but averaging about 0.75 per cent). The foreign water is not removed by keeping the precipitate over concentrated sulfuric acid. Although drying the precipitate at 105° C. or above gives better results, the method is not to be recommended unreservedly, because the dried monohydrate may still contain foreign water; in some cases the amount is very small. The results of drying at 105° C. are variable, the amount of foreign water retained apparently depending upon the relative humidity of the atmosphere. Another objection to drying at 105° C. or above is the possibility of loss of hydrate water. At 100° to 105° C. the monohydrate does not decompose easily under the conditions of humidity usually obtaining, but one cannot be certain that the product weighed is actually the monohydrate. At 110° to 120° C. the monohydrate can easily lose essential water in a dry atmosphere.

If calcium oxalate is precipitated in cold solution in the presence of acetic acid (to prevent the formation of basic oxalate) by the sudden addition of ammonium oxalate, and the mixture is then digested for 20 hours near the boiling point, the product is but slightly hygroscopic and contains relatively little occluded water after air-drying (Nos. 8 and 9, Table I). Precipitation under these conditions gives a precipitate containing a high proportion of the di- and trihydrates of calcium oxalate which are unstable in hot solution. Accordingly on digestion the precipitate recrystallizes and relatively "perfect" crystals of monohydrate are obtained which retain only small amounts of foreign water (*6*). However, the method suffers from the practical disadvantage that the small size of the final crystals tends to make filtration slow.

A second and better method of obtaining a precipitate of calcium oxalate monohydrate containing but a small amount of foreign water consists in precipitation by the method of Willard and Chan (*9*). From the results of determinations 1, 2, and 3 in Table II it will be seen that the neutralization of the acid solution by the hydrolysis of urea must not take place too rapidly. When 10 or 15 grams of urea are used in 200 ml. of solution containing 5 ml. of concentrated hydrochloric acid and the neutralization is completed in 1 to 2 hours, the results are high to the extent of 0.2 to 0.5 per cent

TABLE II. $CaC_2O_4.H_2O$ AS A WEIGHING FORM FOR CALCIUM

(Precipitated by method of Willard and Chan)

No.	Conditions of Precipitation	$CaC_2O_4.H_2O$ Taken	Temperature of Drying	Time	$CaC_2O_4.H_2O$ Found	Error		$CaCO_3$ Found	Error	
		Gram	*° C.*	*Hours*	*Gram*	*Mg.*	*%*	*Gram*	*Mg.*	*%*
1	1.0 gram of $(NH_4)_2C_2O_4.H_2O$ and 10 grams of urea added to 200 ml. of Ca solution containing 5 ml. concentrated hydrochloric acid. Heated near boiling point for 2 hrs. Allowed to cool 30 min. before filtration	0.6796	25	...	0.6809	+1.3	+0.19			
			25 (R. H. = 85%)	18	0.6808	+1.2	+0.18			
			105	5	0.6799	+0.3	+0.04			
			105	40	0.6792	−0.4	−0.06			
			25 (R. H. = 85%)	23	0.6797	+0.1	+0.01			
			25 (R. H. = 85%)	48	0.6798	+0.2	+0.03			
			120	24	0.625	−5.5	−8.1			
			25 (R. H. = 85%)	48	0.6766	−3.0	−0.44			
			25 (R. H. = 85%)	48	0.6790	−0.6	−0.09			
			25 (R. H. = 85%)	48	0.6798	+0.2	+0.03			
			25 (R. H. = 85%)	24	0.6798	+0.2	+0.03	....	...	−0.09[a]
2	As in (1) except neutralization completed in 1.25 hrs. Cooled to room temperature before filtration	0.3617	25		0.3633	+1.6	+0.44			
			25[a]	20	0.3630	+1.3	+0.36			
			105	2	0.3622	+0.5	+0.14			
			105	48	0.3618	+0.1	+0.03	0.2476	−0.2	−0.08
3	As in (1) except 15 grams of urea. Neutralization completed in 1.25 hrs. Cooled to room temperature before filtration	0.7217	25		0.7255	+3.8	+0.53			
			105	1	0.7244	+2.7	+0.37			
			105	1	0.7240	+2.3	+0.32			
			105	22	0.7224	+0.7	+0.10			
			105	20	0.7180	−3.7	−0.51			
			105	2	0.7200	−1.7	−0.24			
			105	20	0.7216	−0.1	−0.01			
			120	4	0.702	−20	−2.8	0.4943	−0.1	−0.02
4	1.0 gram of $(NH_4)_2C_2O_4.H_2O$ and 5 grams of urea added to 200 ml. of Ca solution containing 5 ml. of concentrated hydrochloric acid. Digested overnight at 80°–90°. Cooled to room temperature before filtration	0.7220	25		0.7223	+0.3	+0.04			
			105	1	0.7218	−0.2	−0.03			
			105	2.5	0.7214	−0.6	−0.08	0.4944	−0.2	−0.04
5	As in (4)	0.6139	25		0.6137	−0.2	−0.03			
			25 (R. H. = 85%)	48	0.6135	−0.4	−0.07	....	...	+0.03[b]
6	As in (4)	0.3615	25		0.3621	+0.6	+0.17			
			105	1	0.3616	+0.1	+0.03			
			105	1	0.3615	0.0	0.00			
			105	1	0.3614	−0.1	−0.03	0.2474	−0.2	−0.08
7	As in (4)	0.3617	25		0.3622	+0.5	+0.14			
			25 (R. H. = 85%)	1.5	0.3622	+0.5	+0.14			
			105	1	0.3618	+0.1	+0.03	0.2475	−0.3	−0.12
			25 (R. H. = 85%)	1	0.3621	+0.4	+0.11			
8	As in (4)	0.1448	25		0.1457	+0.9	+0.62			
			105	1	0.1453	+0.5	+0.35	0.0992	0.0	0.0
9	As in (4)	0.1449	25		0.1449	0.0	0.0			
			105	2.5	0.1444	−0.5	−0.35	....	...	....
10	As in (4)	0.1449	25		0.1455	+0.6	+0.41			
			25 (R. H. = 85%)	20	0.1456	+0.7	+0.48			
			105	1	0.1452	+0.3	+0.21			
			25	24	0.1456	+0.7	+0.48	....	...	+0.68[b]
11	As in (4)	0.1499	25		0.1500	+0.1	+0.07			
			25 (R. H. = 85%)	48	0.1498	−0.1	−0.07			
			25 (R. H. = 85%)	170	0.1497	−0.2	−0.13	....	...	+0.16[b]
12	As in (4)	0.1363	25		0.1368	+0.5	+0.37			
			25 (R. H. = 85%)	24	0.1369	+0.6	+0.44			
			120	24	0.121	...	....			
			25 (R. H. = 85%)	48	0.1369	+0.6	+0.44	....	. .	+0.41[b]
13	As in (4)	0.0722	25		0.0724	+0.2	+0.3			
			105	2.5	0.0719	−0.3	−0.4	....	. .	....
14	As in (4) except 0.30 gram of $(NH_4)_2C_2O_4.H_2O$	0.1705	25		0.1710	+0.5	+0.29			
			25 (R. H. = 85%)	20	0.1708	+0.3	+0.18			
			105	1	0.1703	−0.2	−0.12	....	.	....
15	1.0 gram of ammonium oxalate and 5 grams of urea added to 200 ml. of Ca solution, acidified with 5 ml. of hydrochloric acid, containing 0.10 gram of sodium chloride. Digested 18 hrs. (80–90°)	0.3616	25		0.3631	+1.5	+0.41			
			105	2	0.3628	+1.2	+0.33			
			105	1	0.3628	+1.2	+0.33	0.2481	+0.4	+0.16
16	As in (15) except 0.03 gram of NaCl	0.3615	25		0.3623	+0.8	+0.22			
			25 (R. H. = 57%)		0.3624	+0.9	+0.25			
			105	1	0.3622	+0.7	+0.19			
			105	3	0.3619	+0.4	+0.11	0.2475	−0.1	−0.04
17	1.0 gram of ammonium oxalate and 5 grams of urea added to 200 ml. of Ca solution, acidified with 5 ml. of hydrochloric acid, containing 0.100 gram of $MgC_2O_4.2H_2O$. Digested 24 hrs. at 80–90°. Filtered while still hot	0.3615	25		0.3616	+0.1	+0.03			
			105	1	0.3610	−0.5	−0.14			
			105	3	0.3610	−0.5	−0.14	0.2471	−0.5	−0.20
18	As in (17) except solution cooled to room temperature before filtration	0.3617	25		0.3621	+0.4	+0.11			
			25 (R. H. = 85%)	2	0.3619	+0.2	+0.06			
			105	2	0.3612	−0.5	−0.14			
			105	3	0.3607	−1.0	−0.28			
			25 (R. H. = 85%)	48	0.3616	−0.1	−0.03			
			105	3	0.3600	−1.7	−0.47	0.2478	0.0	0.00

[a] Over anhydrous calcium chloride.
[b] From permanganate titration.

after air-drying. On the other hand, when 5 grams of urea are taken for 200 ml. of solution containing 5 ml. of concentrated hydrochloric acid and the hydrolysis is allowed to proceed overnight at 80° to 90° C. the error is reduced to +0.2 per cent or less for amounts of calcium equivalent to 0.25 to 0.5 gram of calcium carbonate. For smaller amounts of calcium (0.05 to 0.1 gram of calcium carbonate) under these conditions there is a distinct tendency for the positive error to increase, which is attributable mainly to coprecipitation of oxalic acid; in one case (No. 8) the error amounted to +0.6 per cent. The oxalate content of precipitates 10, 11, and 12 as determined by permanganate titration indicates that there is appreciable coprecipitation of ammonium oxalate, acid calcium oxalate, or oxalic acid with the smaller amounts of calcium oxalate, under the conditions of precipitation used. The larger amounts of precipitate (Nos. 1 and 5) show a normal oxalate content.

If the precipitate is dried at 105° C. the results are closer to the theoretical than when the precipitate is air-dried, but this method is not recommended because of the possibility of loss of hydrate water (see No. 3, Table II, in which some decomposition took place after long heating at 105° C.).

The coprecipitation of sodium is marked (Table II, No. 15) and only small amounts (No. 16) may be present unless a reprecipitation is made. Magnesium is not appreciably coprecipitated when present in small amounts, as shown by determinations 17 and 18 of Table II; these results indicate that even if a precipitate of calcium oxalate is badly contaminated by magnesium it can be freed from the latter element if the reprecipitation is made by the urea-hydrolysis method.

Recommended Procedure

Prepare a solution of calcium salt containing the equivalent of 0.2 to 0.5 gram of calcium carbonate in 175 to 200 ml. of solution, add 5 ml. of concentrated hydrochloric acid, and heat nearly to the boiling point. Add 1.0 gram of ammonium oxalate monohydrate dissolved in approximately 20 ml. of hot water (no precipitate should form) and then 5.0 grams of reagent quality urea dissolved in a similar volume of cold water. After mixing, heat at 80° to 90° C. until the solution is distinctly basic to methyl orange (overnight). Cool the solution and collect the precipitate in a porous porcelain, sintered-glass, or Gooch crucible which has been weighed after standing 10 to 15 minutes in the air. Wash the precipitate with small portions of cold water and then with three or four 2-ml. portions of reagent quality acetone. Draw air through the crucible for 5 to 10 minutes and weigh. It is well to let the crucible stand on the balance pan for 10 to 15 minutes after the first weighing, and then to reweigh to be certain that the weight is constant.

In the presence of appreciable amounts of sodium or magnesium the first precipitation of calcium oxalate may be made by the ordinary method, and after washing with dilute ammonium oxalate solution the precipitate may be dissolved in hydrochloric acid and reprecipitated as described in the previous paragraph; in this case the amount of ammonium oxalate added in the final precipitation need not be more than 0.2 to 0.3 gram.

Summary

Calcium oxalate monohydrate precipitated from neutral or ammoniacal solutions, or by neutralizing a hydrochloric acid solution with ammonia, retains foreign water after washing with acetone or alcohol and ether, and air-drying. Drying the precipitate at 105° C. and above yields results closer to the theoretical than does air-drying at room temperature, but this method of drying cannot be recommended without reservation because considerable amounts of water may still be retained, constant weight is not always easily attained, and the precipitate may lose monohydrate water in a dry atmosphere.

If calcium oxalate is precipitated slowly from an acid solution by gradual neutralization of the acid by the hydrolysis of urea, it may be weighed as the monohydrate after washing with acetone. The solution, containing 5 ml. of concentrated hydrochloric acid per 200 ml., is treated with 5 grams of urea and digested at 80° to 90° C. until the acid has been neutralized. In this manner results accurate to approximately 0.2 per cent can be obtained with 0.2 to 0.5 gram of calcium carbonate; with amounts of calcium corresponding to 0.05 to 0.1 gram of calcium carbonate results tend to be high.

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RECEIVED July 29, 1938.

Glass Liner for High-Pressure Hydrogenation Bomb

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THE glass container shown in the accompanying figure has proved satisfactory as a liner for high-pressure hydrogenation bombs. It has been constructed in net capacities of 10 and 30 cc., and the design is feasible for larger volumes.

The body of the liner should fit the bomb snugly and, as shown, the joint should be rimless. A steel compression spring maintains closure and prevents rotation of the liner. Correct alignment of the gas inlet tube is assured by a mark on the rim of the male member of the joint which corresponds with the upper end of the constricted tube. A circular mark around the body of the tube about 5 mm. below the gas inlet serves as a reference point for calibration.

The authors have not succeeded in repairing broken liners which had been used at high temperatures and high pressures of hydrogen, owing to apparent absorption and retention of gas, which is liberated at glass-working temperatures causing the glass to froth.

RECEIVED November 19, 1938.

Determination of Potassium with Hexanitrodiphenylamine (Dipicrylamine) Reagent

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Gravimetric and volumetric procedures are given for the determination of macro- and microquantities of potassium as dipicrylaminate; and a colorimetric procedure for the determination of microquantities (10 to 100 gamma).

HEXANITRODIPHENYLAMINE or dipicrylamine is a relatively weak acid which is practically insoluble in water. Its potassium, ammonium, rubidium, and cesium salts are slightly soluble in water, have an orange-red to red color, and are crystalline. Poluektoff (*7*) was the first to use a solution of the sodium salt of dipicrylamine as a reagent for the detection of potassium. Van Nieuwenburg and van der Hoek (*6*) described the crystal habit of the above salts and recommended the reagent for the microchemical detection of potassium, even in the presence of cesium.

Sheĭntzis (*10*) found that in addition to the above-named metals thallous thallium, beryllium, zirconium, lead, and mercuric mercury gave colored crystalline precipitates with the sodium salt of dipicrylamine; while aluminum, ferric iron, chromic chromium, nickel, cobalt, copper, bismuth, vanadium, titanium, thorium, and mercurous mercury gave amorphous precipitates. The reagent has an alkaline reaction and the last-mentioned group of cations should yield a precipitate which may consist of the hydrous oxide or some basic salt.

Feigl (*3*) includes the sodium salt of dipicrylamine in his review of spot tests for potassium. According to the authors' experience filter paper impregnated with the reagent is very suitable for the detection of potassium, even in the presence of much sodium and other cations.

Winkel and Maas (*11*) give procedures for the quantitative determination of potassium, either by weighing the precipitate or by conductometric titration of a solution of the precipitate in a mixture of acetone and water. Portnov and Afanas'ev (*8*) using the titration method report an accuracy of 0.5 to 1.5 per cent. Recently Kielland (*5*) has applied the reagent to the colorimetric determination of potassium in fertilizers by using a gradation photometer.

Using 10 mg. of potassium and following the directions of Winkel and Maas for gravimetric determination, the authors found that results were consistently about 3 per cent low. This prompted them to make a systematic study of the sources of error, which led to the development of satisfactorily accurate procedures for the determination of macro- and microquantities of potassium. The solubility of the potassium salt in water and in an excess of reagent is appreciable and varies strongly with the temperature. The solutions are decomposed by acid with a separation of the free amine. The potassium salt is freely soluble in acetone and also soluble in ether, ethanol, and methyl amyl ketone, but insoluble in chloroform, dichloroethane, carbon tetrachloride, and benzene. The free amine is very slightly soluble in water (light yellow color), insoluble in dilute mineral acids, chloroform, carbon tetrachloride, dichloroethane, and benzene, but soluble in acetone, ether, and methyl amyl ketone. Indications have been obtained that the potassium salt does not behave as an ideally strong electrolyte in water and in organic solvents.

The main source of error was found in the relatively great solubility of the potassium dipicrylaminate in water and in an excess of reagent. A saturated solution of the salt in water was prepared at 25° and 0° C. By colorimetric analysis the solubilities were found to be 0.88 and 0.073 gram per liter, respectively. In order to get an idea of the losses by solubility under conditions at which a quantitative determination may be carried out, the following experiments were made:

a. An accurately weighed amount of the potassium salt (about 0.1 gram) was placed in a sintered-glass crucible and 50 ml. of 1.5 per cent magnesium dipicrylaminate solution delivered from a pipet were drawn through slowly at room temperature. The precipitate left was washed with 0.5 ml. of ice water, dried at 110° C., and weighed. The loss in weight corresponded to 0.14 gram per liter of reagent at room temperature (25 ± 1° C.).

b. Seventy-five milliliters of a 0.5 per cent solution of magnesium dipicrylaminate were added to a beaker which had been weighed together with 0.1000 gram of the potassium salt and a small porcelain filter stick. The solution was stirred at frequent intervals for 2.5 hours. The liquid was removed by suction, etc. The loss in weight corresponded to 0.25 gram per liter of 0.5 per cent reagent.

c. Experiment *b* was repeated, but the liquid was stirred with the salt at 0° C. The loss in weight was 0.028 gram per liter of 0.5 per cent reagent.

d. After preparing a saturated solution as mentioned under *b*, the beaker and its contents were cooled to 0° C. and left at this temperature for 2.5 hours. The loss in weight was 0.05 gram per liter of 0.5 per cent reagent.

These experiments show that the solubility increases about 10 times when the temperature is raised from 0° to 25° C. In the first series of experiments a suitable excess of reagent was added to the potassium solution, and the suspension was cooled and kept in ice water until no more precipitate separated. The precipitate was then collected on a sintered-glass crucible, washed with 1 ml. of ice water, then with a saturated solution of the potassium salt at 0° C., and finally with 1 ml. of ice water. The precipitate was dried and weighed. Using 10 mg. of potassium the results were relatively 2 to 3 per cent low. That these low results are to be attributed mainly to a rise in temperature of the wash solutions in the crucible during the washing is evidenced by the following experiments:

About 0.1000 gram of the potassium salt was placed on a sintered-glass crucible and washed with gentle suction with 25 ml. of ice water delivered dropwise from a pipet. The time of washing was about 2 minutes and the loss in weight corresponded to 0.26 gram per liter, whereas the solubility at 0° C. was found to be 0.073 gram per liter. In another experiment a weighed amount of the salt was washed with 75 ml. of the saturated solution of the potassium salt at 0° C. The loss in weight was 0.14 gram per liter. In order to limit the loss by solubility to a minimum, the suspension of the potassium salt in an excess of reagent must be cooled to 0° C., filtered, and washed with suitable wash liquids at this temperature.

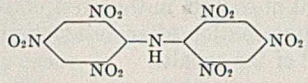
Other errors, due to coprecipitation with the potassium salt, are discussed below.

Gravimetric Determination of Potassium

The potassium is precipitated as dipicrylaminate from a neutral or slightly alkaline aqueous solution by addition of an excess of magnesium or sodium dipicrylaminate. The mixture is cooled

to 0° C. and filtered with the aid of a filter stick, washed with ice water and an aqueous solution of potassium dipicrylaminate which is saturated at 0° C., dried at 110° C., and weighed.

MATERIALS USED. Dipicrylamine



can be prepared according to the directions of Austens (*2*) or Hoffman and Dame (*4*) or by direct nitration of diphenylamine (*1*).

A product suitable for analytical purposes can be obtained from the Eastman Kodak Company, Rochester, N. Y., and was used in most of the work. It can be recovered easily by acidifying the filtrate containing the excess of reagent, and from the weighed potassium salt, by dissolving the latter in a little acetone, diluting with water, and acidifying. Caution should be observed with the acid and the reagent, as when they come in contact with the skin they may cause blisters, resembling burns that cause intense itching and heal slowly. The reagent does not cause immediate discomfort when it gets on the skin and several days elapse before the blisters appear.

The other materials used were C. P. products which were purified by standard methods when necessary. The salts used in testing the effect of their presence upon the determination of potassium were found to contain less than 0.01 mg. of potassium per gram, as indicated by the sodium cobaltinitrite test.

MAGNESIUM DIPICRYLAMINATE REAGENT: 3 per cent. Twelve grams of dipicrylamine are mixed with 5 grams of magnesium oxide and the mixture is transferred with 400 ml. of water to a 500-ml. Erlenmeyer flask. The solution is stirred well, allowed to stand for 15 to 20 hours, and filtered. The concentration of a reagent prepared in this manner can be determined by evaporating 5 ml. to dryness and weighing the residue. It was found to be 3 per cent or 0.066 *N* with respect to magnesium dipicrylaminate. This reagent is referred to as 3 per cent reagent.

Magnesium oxide is preferred to magnesium carbonate, as the former dissolves the amine more rapidly. Heat should not be applied in the preparation of the reagent, because solutions prepared in this way tend to deposit a solid on standing. If the reagent becomes turbid, it should be filtered before use.

SODIUM DIPICRYLAMINATE SOLUTIONS: 3 per cent. Although the magnesium reagent is used in most of the determinations, the sodium reagent may be of advantage in the presence of anions which form a precipitate with magnesium. The sodium reagent is prepared by mixing the amine with a slight excess of sodium carbonate and diluting with water to give a solution which is 3 per cent in sodium dipicrylaminate.

WASHING SOLUTION 1. Distilled water cooled to 0° C. by placing 50 ml. in a 100-ml. Erlenmeyer flask in an ice bath.

WASHING SOLUTION 2. A saturated solution of potassium dipicrylaminate in water at 0° C. is prepared by adding an excess of the potassium salt to water in a beaker at room temperature. The beaker is placed in ice water and after standing at least a few hours the wash solutions can be drawn off through a filter stick or by pipetting off the clear supernatant liquid.

STANDARD POTASSIUM CHLORIDE SOLUTION. A solution of recrystallized and dried C. P. potassium chloride is prepared, containing 10 mg. of potassium per 5 ml. of solution.

PROCEDURE. An ordinary 30-ml. porcelain crucible containing a thin glass stirring rod and a small Emich (*9*) porcelain filter stick are weighed together on an analytical balance. (The diameter of the plate of the filter sticks used was 10 mm. and their height 50 mm. They may be purchased from the Fish-Schurmann Corporation, 250 East 43rd St., New York, N. Y.) A known amount of the sample is placed in the crucible and the volume so adjusted that the solution contains about 2 mg. of potassium per milliliter. If the solution is acid it is neutralized with sodium hydroxide until neutral to thymol blue; if it is alkaline it is neutralized with hydrochloric acid using the same indicator. With constant stirring 50 to 100 per cent excess of the 3 per cent magnesium reagent (for 10 mg. of potassium 7 ml. of reagent are used) is added dropwise to precipitate the potassium. The crucible containing the solution and stirring rod is cooled for at least 15 minutes in ice water and then placed in a shallow dish filled with ice water. The filter stick is mounted just above the bottom of the crucible as indicated in Figure 1, the supernatant liquid removed, and the precipitate sucked as dry as possible. The precipitate is washed once with 1 ml. of washing solution 1 (to avoid precipitation of the potassium salt by common-ion effect) then with three to four 1-ml. portions of washing solution 2, and finally with 0.5 ml. of washing solution 1. The filter stick is disconnected and placed in the crucible with the stirring rod. The outside of the crucible is wiped clean, and the crucible and contents are dried at 110° C. for 1 hour, cooled in a desiccator, and weighed. The weight of the precipitate multiplied by 0.08194 yields the amount of potassium.

TABLE I. GRAVIMETRIC DETERMINATION OF POTASSIUM

Potassium Taken	Weight of Precipitate	Potassium Found	Relative Error
Mg.	*Gram*	*Mg.*	*%*
20.00	0.2439	19.98	−0.1
20.00	0.2432	19.93	−0.4
10.00	0.1215	9.95	−0.5
10.00	0.1217	9.97	−0.3
10.00	0.1220	9.99	−0.1
10.00	0.1217	9.97	−0.3
10.00	0.1218	9.98	−0.2
10.00	0.1222	10.01	0.1
10.00	0.1217	9.97	−0.3
4.95	0.0605	4.96	0.2
4.99	0.0611	5.006	0.3
1.118	0.0137	1.112	−0.5
1.006	0.0123	1.007	0.1
1.000[a]	0.01202	0.984	−1.6
1.000[a]	0.01199	0.982	−1.8

[a] In these cases the potassium salt was dissolved in 5 ml. of water and precipitated with 4 ml. of reagent.

As shown in Table I, the procedure gives satisfactory results with amounts of potassium varying between 5 and 20 mg. In the experiments with 1 mg. of potassium a semimicrobalance was used. Even with this small amount of potassium the results were gratifying.

Effect of Foreign Ions

No attempt has been made to determine potassium in the presence of rubidium and cesium, which also yield slightly soluble dipicrylaminates.

AMMONIUM. Ammonium salts have to be removed, as ammonium dipicrylaminate is slightly soluble. This can be done easily by boiling the solution with a slight excess of magnesium oxide until the vapors do not change the color of sensitive litmus paper. The solution is filtered, the precipitate washed, and the filtrate evaporated to the desired volume. Naturally, the magnesium oxide should be tested for the presence of potassium and a correction should be made, if necessary. In the determination of 10 mg. of potassium by the procedure in the presence of 100 mg. of ammonium added as ammonium sulfate, the results were 0.4 to 0.5 per cent high.

When the ammonium content is not too large, it may be possible to make the ammonium harmless by the addition of

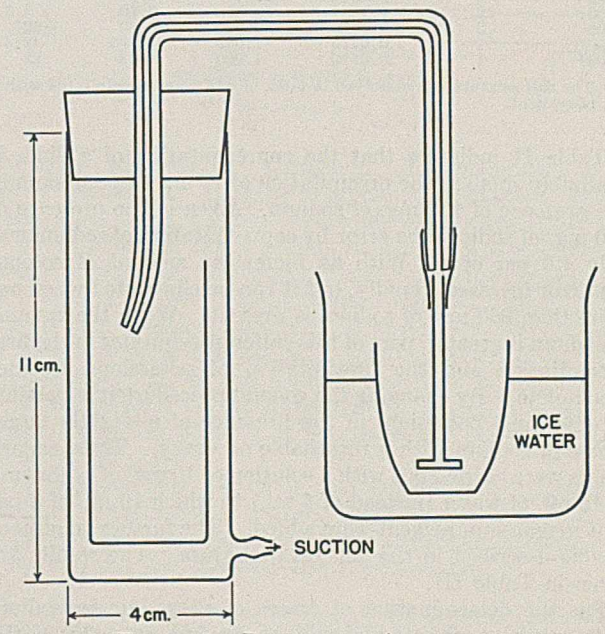


FIGURE 1. DIAGRAM OF APPARATUS

a slight excess of sodium hydroxide, as free ammonia does not give a precipitate. In this case the potassium should be precipitated with the sodium dipicrylaminate solution.

TABLE II. DETERMINATION OF 10.00 MG. OF POTASSIUM IN THE PRESENCE OF SODIUM

Sodium Added	Number of Precipitations	Weight of Precipitate	Potassium Found	Relative Error
Mg.		*Mg.*	*Mg.*	%
50	1	0.1213	9.94	−0.6
55	1	0.1220	10.00	0.0
81	1	0.1217	9.97	−0.3
96	1	0.1218	9.98	−0.2
177	1	0.1240	10.16	1.6
220	1	0.1305	10.70	7.0
220	1	0.1308	10.71[a]	7.1
300	1	0.1452	11.90	19.0
220	2	0.1221	10.00	0.0
240	2	0.1212	9.93	−0.7
300	2	0.1184	9.70	−3.0
500	2	0.1184	9.70	−3.0
600	2	0.1160	9.50	−5.0

[a] Reagent added to boiling solutions and mixture cooled. Apparently, temperature of precipitation does not affect amount of coprecipitation.

SODIUM. As the determination of potassium in the presence of sodium is of great practical importance, the effect of sodium was investigated in a fairly extensive way. Sodium was added to the solution in the form of chloride, nitrate, or sulfate; the type of anion used was found to have no effect. Some of the results are given in Table II.

In case double precipitation was used the original precipitate was filtered as described in the general procedure. After removing the excess of reagent, the precipitate was not washed but dissolved in 3 ml. of acetone. The filter stick was washed dropwise with acetone and removed from the crucible. The solution in the crucible was diluted with 5 ml. of water and 3 ml. of reagent were added (in the first precipitation 7 ml. of reagent were used). The mixture was heated on the steam bath until no odor of acetone was noticeable, cooled, and treated further as described in the procedure.

TABLE III. DETERMINATION OF POTASSIUM IN THE PRESENCE OF LARGE AMOUNTS OF SODIUM

(Modified procedure)

Sodium Added	Number of Precipitations	Weight of Precipitate	Potassium Taken	Potassium Found	Relative Error
Mg.		*Mg.*	*Mg.*	*Mg.*	%
0.0	1	0.1220	10.00	9.99	−0.1
0.0	2	0.1216	10.00	9.96	−0.4
108	1	0.1224	10.00	10.03	0.3
300	2	0.1207	10.00	9.89	−1.1
400	2	0.1184	10.00	9.70	−3.0
800	2	0.0830	10.00	6.80	−32
80[a]	1	0.0119	1.000	0.975	−2.5
200[a]	1	0.0150	1.000	1.23	23

[a] The salt mixture was dissolved in 5 ml. of water and precipitated with 4 ml. of reagent.

Table II indicates that the coprecipitation of sodium is negligibly small in the precipitation of 10 mg of potassium in the presence of 100 mg. of sodium. Even in the presence of 180 mg. of sodium the error by coprecipitation of sodium was only 1.6 per cent. With an increasing amount of sodium the error increases rapidly, but it can be eliminated when not more than 250 mg. of sodium is present. When the amount of sodium is greater, part of this cation precipitates in the first precipitation and the precipitation of potassium becomes incomplete. By changing the general procedure it is possible to determine potassium in the presence of a slightly larger excess of sodium with a reasonable accuracy. Thus, experiments were carried out with a solution of 10 mg. of potassium in 15 ml. of water (instead of 5 ml.) to which 10 ml. of 3 per cent magnesium reagent were added. The further treatment was as described in the general procedure. The results are given in Table III.

For the determination of traces of potassium in sodium salts special procedures should be worked out. From the results in Table III it is seen that 1 mg. of potassium can be determined in the presence of 80 mg. of sodium with an accuracy of 2.5 per cent. When the ratio becomes still less a separation of the potassium as cobaltinitrite is advisable. The small amount of sodium in the precipitate will not interfere with the determination after destruction of the precipitate and removal of the cobalt.

TABLE IV. DETERMINATION OF POTASSIUM IN THE PRESENCE OF LITHIUM, MAGNESIUM, CALCIUM, AND BARIUM

(Potassium taken, 10.00 mg.)

Foreign Ion Present	Amount Added	Weight of Precipitate	Potassium Found	Relative Error
	Mg.	*Gram*	*Mg.*	%
Li	12	0.1218	9.98	−0.2
	50	0.1235	10.12	1.2
	109	0.1261	10.33	3.3
Mg	10	0.1219	9.99	−0.1
	50	0.1219	9.99	−0.1
	100	0.1217	9.97	−0.3
	200	0.1230	10.08	0.8
Ca	32	0.1221	10.00	0.0
	84	0.1226	10.04	0.4
	170	0.1230	10.08	0.8
	320	0.1284	10.52	5.2
Ba	6	0.1293	10.6	6.0
	27	0.1606	13.2	32.0
	52	0.1961	16.1	61.0

LITHIUM, MAGNESIUM, CALCIUM, AND BARIUM. In Table IV it is shown that the procedure gives good results in the presence of relatively large amounts of magnesium, lithium, and calcium. In the determination of 1.000 mg. of potassium in the presence of 100 mg. of calcium the error was 0.4 per cent. Barium interferes, and should be removed. Table IV shows that the error increases linearly with the barium concentration in the solutions, indicating a mixed crystal formation of potassium and barium dipicrylaminates.

TABLE V. DETERMINATION OF 10.00 MG. OF POTASSIUM IN THE PRESENCE OF METALS GIVING A PRECIPITATE WITH THE REAGENT

Metals Added	Magnesium Oxide Used	Potassium Found	Relative Error
	Gram	*Mg.*	%
	0.2	9.95	−0.5
	0.2	9.98	−0.2
	2.0	9.78	−2.2
10 mg. each of Fe^{+++}, Al, Cr^{+++}, Zn, Ni, Cu, Co, Zn	0.2	10.03	+0.3
	0.2	10.07	+0.7
100 mg. each of above metals[a]	2.0	9.90	−1.0
	2.0	9.82	−1.8
	2.0[b]	10.12	+1.2

[a] Without addition of potassium no precipitate of potassium dipicrylaminate was found after separation.

[b] 1 gram of magnesium sulfate was added to mixture before addition of magnesium oxide.

Experiments were also carried out with a mixture containing 10 mg. of potassium, 100 mg. of sodium, 20 mg. of lithium, and 100 mg. of magnesium. The errors found fluctuated between −0.3 and +0.3 per cent.

METALS WHICH FORM A PRECIPITATE IN ALKALINE MEDIUM interfere with the potassium determination. As relatively large amounts of magnesium do not interfere (see Table IV), the use of magnesium oxide for the removal of these cations is suggested. The results reported in Table V were obtained in the following way:

A solution of the chlorides of the metals to be separated was diluted to approximately 50 ml. in a 150-ml. beaker. A measured volume of potassium chloride solution containing 10.00 mg. of potassium and the indicated excess of magnesium oxide were added. After boiling gently for 10 minutes the suspension was allowed to cool, filtered, and the precipitate washed. The combined filtrate and washings were evaporated to about 10 ml. and transferred to a 30-ml. crucible in which the solution was evaporated to 5 ml. The potassium was precipitated by the regular procedure.

The results are satisfactory. When the ratio of potassium to other cations (100 mg. of each) is unfavorable, apparently some potassium is absorbed by the precipitate of hydrous ox-

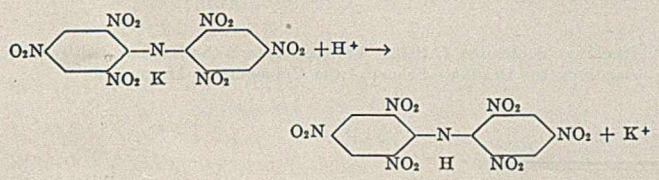
ides. This adsorption can be decreased by the addition of magnesium sulfate, in which case slightly high results were found, undoubtedly due to coprecipitation of magnesium with the potassium dipicrylaminate. Probably a reprecipitation of the latter would improve the results. However, the authors have not attempted to find suitable procedures for all cases which may occur.

PHOSPHATE. The presence of phosphate interferes when the magnesium reagent is used; the 3 per cent sodium reagent should be used instead. Determinations were made with a mixture of 10.00 mg. of potassium and 90 mg. of phosphate (as Na_2HPO_4) using the sodium reagent. The phosphate was found to cause no interference; in the absence of phosphate the results fluctuated between −0.3 and +0.3 per cent, and in the presence of phosphate between −0.3 and 0.0 per cent. When other anions are present which precipitate with magnesium, the sodium reagent should be applied.

Acidimetric Determination

Although the gravimetric procedure is simple and rapid, the volumetric procedure may have advantages in routine analyses.

PROCEDURE. The potassium is precipitated and washed as in the gravimetric procedure. The receptacle used to collect the filtrate (see Figure 1) is replaced by a clean one. With the filter stick connected with the suction apparatus, acetone is added dropwise down the sides of the crucible and the solution is drawn over into the receptacle. After all the precipitate in the crucible and on the filter stick has dissolved and the acetone remains colorless, the suction is turned off and the receptacle is removed. The acetone solution is diluted with 5 to 10 ml. of water, and heated to ensure complete solution of the potassium salt. A measured excess of standard acid is added and the receptacle is placed on a steam bath to coagulate the precipitated dipicrylamine and to remove the acetone. When no odors of acetone are noticeable, the mixture is cooled in ice water and the amine is filtered off on a sintered-glass crucible and washed with ice water. The combined filtrate and washings are heated to boiling to expel carbon dioxide and, while hot, titrated with standard sodium hydroxide using bromothymol blue as indicator. The amount of acid used is equivalent to the amount of potassium in the precipitate; 1 ml. of 0.1 *N* acid corresponds to 3.91 mg. of potassium.



The suspension of the amine is cooled and the washing is carried out with ice water in order to minimize the amount of amine going into solution. In blank experiments 50 ml. of ice-cold water were drawn through a washed precipitate of the amine. The filtrate had a slightly yellow color but required only 0.05 ml. of 0.01 *N* sodium hydroxide to change the color of the indicator.

TABLE VI. GRAVIMETRIC AND VOLUMETRIC DETERMINATION OF 10.00 MG. OF POTASSIUM

Foreign Ion Present	Amount of Foreign Ion	Potassium Found Gravimetrically	Relative Error	Potassium Found Volumetrically	Relative Error
	Mg.	*Mg.*	%	*Mg.*	%
...	..	10.01	+0.1	9.99	−0.1
...	..	9.98	−0.2	9.97	−0.3
...	..	9.95	−0.5	10.02	+0.2
...	..	9.94	−0.6	9.95	−0.5
Na	50	9.97	−0.3	10.00	0.0
Na	96	9.98	−0.2	10.04	+0.4
Ca	18	10.00	0.0	10.02	+0.2

In the experiments with 10.00 mg. of potassium reported in Table VI, 5 ml. of 0.1 *N* hydrochloric acid were used to precipitate the amine and the back-titration was made with 0.035 *N* sodium hydroxide. In these experiments the potassium dipicrylaminate was first weighed and then determined volumetrically.

Experiments have also been carried out by an indirect method. A measured excess of standard reagent was added to the solution of the potassium salt and the amount of dipicrylaminate left in the filtrate and washings was determined acidimetrically. The reagent was standardized under similar conditions. This method may be of practical importance with larger amounts of potassium (20 mg. or more), but is not recommended for the determination of smaller amounts, as a fairly large excess of reagent has to be used to ensure complete precipitation of potassium. Working with 10 mg. of potassium results were accurate to about 1 per cent.

Colorimetric Determination

The colorimetric method is particularly suitable for the determination of microquantities of potassium. The aqueous solutions vary in color from an orange-red at saturation to a light yellow at low concentrations. The light absorption of slightly alkaline aqueous solutions of the potassium salt in water at concentrations varying between 0.25 and 3.0 mg. of salt per liter was measured in a B. Lange photoelectric colorimeter using a blue filter. In Figure 2 are plotted the values of the logarithm of the percentage of transmitted light $\log (I/I_0 \times 100)$ against the concentration, and it is seen that Beer's law does not hold for aqueous solutions of potassium dipicrylaminate. Therefore, the ordinary colorimeter is not suitable for the colorimetric determination of potassium. However, one can use the photoelectric colorimeter and make a calibration curve or apply the equicolor method as in a Nessler determination or a colorimetric titration. The former method is the simplest.

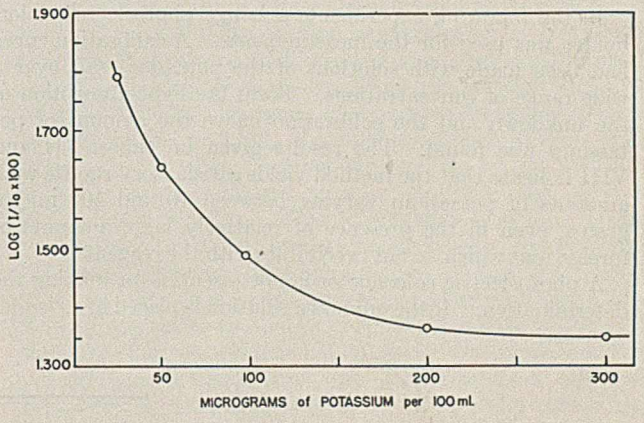


FIGURE 2

Aqueous solutions of potassium dipicrylaminate are slightly hydrolyzed. In order to repress the hydrolysis completely, 1 ml. of 0.1 *N* sodium hydroxide was always added to 100 ml. of solution. More sodium hydroxide does not affect the light absorption. The aqueous solutions of the potassium salt are quite stable. The light absorption was found unchanged after a week of standing. It was hardly affected by the temperature—for example, a solution which absorbed 67.3 per cent at 25° C. was found to absorb 67.4 per cent at 40° C. The color of the dipicrylamine ion is intense; a solution containing 0.02 mg. of potassium salt per 100 ml. is still distinctly yellow.

The largest source of error in the determination of microquantities of potassium is the relatively large solubility. For the quantitative separation of amounts of potassium between 0.1 and 0.01 mg. the following procedure was used.

PROCEDURE. The solution is evaporated to dryness in a 20-ml. porcelain crucible. Three drops of the three per cent magnesium (or, if desirable, sodium) reagent are added to precipitate the

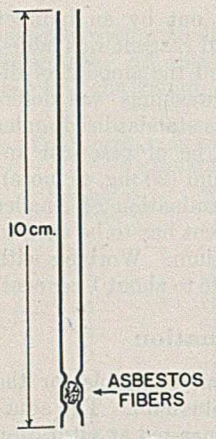


FIGURE 3

potassium, and the crucible is placed in ice water for at least 15 minutes. Without removing the crucible from the ice water the precipitate is collected on a filter stick (cooled in ice water) as shown in Figure 3. This stick consists of a 4-mm. glass tubing containing two constrictions between which asbestos fibers are placed. The mother liquor is removed by suction, and the precipitate is washed with 2 drops of ice water (washing solution 1, gravimetric procedure), then with 7 to 10 drops of washing solution 2, and finally with one drop of ice water. The filter stick is removed from the suction and the longer section of the stick is filled with acetone by means of an eye dropper. The end opposite the asbestos is placed in the mouth and the acetone is blown through the asbestos, collecting the solution in the crucible in which the precipitation has been made. This is repeated until all the precipitate has dissolved and the acetone has become colorless. The acetone solution is then diluted to 100 ml. (or any other desired volume) with water containing 1 ml. of 0.1 *N* sodium hydroxide per 100 ml.

TABLE VII. COLORIMETRIC DETERMINATION OF 10 TO 100 MICROGRAMS OF K^+

K taken, γ	100	100	90	80	70	60	50	40	30	20	20	10
Light absorption, %	67.5	69	67.0	64.8	62.5	59.8	55.8	50.0	44.1	33.0	34.0	18.0
K found, γ	94	100	90	78	68	59	50	39	31	20	21	9
Error, γ	−6	0	0	−2	−2	−1	0	−1	+1	0	+1	−1

In the following experiments a Lange photoelectric colorimeter was used for the measurements. A calibration curve had been made with solutions of the potassium salt over a wide range of concentrations. From the light absorption of the unknown and the calibration curve the amount of potassium was found. The results given in Tables VII and VIII indicate that the method yields satisfactory results with amounts of potassium varying between 10 and 100 micrograms, even in the presence of relatively large amounts of foreign ions which do not precipitate with the reagent.

A photoelectric colorimeter is not essential for making the determination. If the unknown solution is placed in a Nessler tube and its color matched by adding a standard solution of the potassium salt to 0.001 *N* sodium hydroxide in a second tube, the amount of potassium can be calculated in the unknown. As Beer's law does not hold, the volumes when the final comparison is made should be the same. Several determinations made by this method yielded results of the same order of accuracy as obtained with the photoelectric colorimeter.

TABLE VIII. COLORIMETRIC DETERMINATION OF POTASSIUM IN THE PRESENCE OF FOREIGN IONS

Foreign Ion	Amount of Foreign Ion	Potassium Taken	Potassium Found	Error
	Mg	γ	γ	γ
...	...	50	47	−3
...	...	50	48	−2
Na	0.5	50	48	−2
	1.0	50	45	−5
	3.0	50	48	−2
Mg	0.5	50	48	−2
	1.0	50	50	0
	3.0	50	54	+4
Li	1.0	50	50	0
Ca	1.0	50	54	+4
		25	27	+2
Na	3.0	25	36	+11
Mg	1.0	25	29	+4
Li	1.0	25	29	+4
Ca	1.0	25	29	+4

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Colorimetric Determination of Ascorbic Acid

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RECENTLY Basu and Nath (*1*) reported the reduction of 2,6-dichlorophenolindophenol by ferrous salts in the presence of dibasic and hydroxy organic acids. Similarly Lorenz and Arnold (*2*) show the interference of the ferrous ion on the dye. Some time ago, A. J. Lorenz advised the authors that work from the California Fruit Growers Exchange laboratories indicated that "most canned citrus juices showed about 40 p. p. m. tin, and some iron, tin affecting the iodine titration and iron the 2,6 dye." A representative of the National Canners Association has advised that "no chemical preservatives are required and none are ever used" in canned grapefruit juice, and that "heat constitutes the sole means of preservation in canned foods." The statement made by the authors (*3*) "the higher values obtained for the canned sample of grapefruit juice may be due to preservatives" is therefore misleading. The above information is submitted as a possible source for the explanation of the discrepancy observed by them.

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Reaction between Amines and Sodium 1,2-Naphthoquinone-4-Sulfonate

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THE fact that sodium 1,2-naphthoquinone-4-sulfonate yields colored solutions with compounds containing amino groups (*2*, *5*, *9*, *11*, *16*, *17*) constitutes the basis of the Folin method for the colorimetric determination of the amino acid content of blood and urine (*6*, *7*). No quantitative data are available, however, as to the amount of color given by this reagent with various aliphatic and aromatic amines, although these compounds frequently accompany the amino acids in biological media. When this information became of importance in connection with studies involving the concentration of certain nitrogenous constituents in blood and urine, the present investigation was undertaken. Folin's amino acid procedure has enjoyed a wide usage in biochemical fields (*1*, *8*, *12*). Certain divergent findings (*4*, *10*, *15*), however, which Danielson (*3*) contends result from the limited range in true proportionality due to the presence of a strong residual blank, have been reported. Hence Danielson (*3*) utilizes a strongly acid bleaching reagent in his modification of the original Folin method. An investigation of the factors which influence the shade and amount of color given by these procedures with various amines is reported in the present paper.

Material and Procedure

The aliphatic amines were generally analyzed by adding a weighed portion to water and titrating to methyl red with standard acid. The aromatic amines were usually repurified by commonly accepted procedures. A carefully weighed sample of the compound being studied was diluted to a definite volume with distilled water. Occasionally a few drops of acid were necessary to render the compound water-soluble. Aliquot portions of stock solution were diluted with water to yield solutions containing quantities of nitrogen ranging from 0.042 to 0.112 mg. per 5 cc. The standard for comparison in all cases was glycine. For the Folin method the usual stock glycine solution was diluted to give a standard in 0.02 *N* hydrochloric acid, containing 0.07 mg. of nitrogen per 5 cc. The glycine standard for the Danielson method was prepared in 0.014 *N* hydrochloric acid, 5 cc. also containing 0.07 mg. of nitrogen.

Influence of Alkalinity and Acidity

A 5-cc. sample of the glycine standard was made definitely pink to phenolphthalein by the addition of 1 cc. of the sodium carbonate reagent. Two cubic centimeters of the borax solution were used in the Danielson procedure. To a series of tubes each containing 0.07 mg. of amine nitrogen, or a multiple thereof if the nitrogen group of the compound being studied was not completely reactive, varying quantities of the alkalies were added, the contents were diluted to 10 cc. with distilled water, 2-cc. portions of freshly prepared 0.5 per cent quinone reagent were added, and the tubes were stoppered and placed in the dark for about 24 hours. The acid bleaching reagents were then added, and the contents were diluted to 25 cc. and matched against the appropriate glycine standards in the colorimeter.

Although a certain degree of alkalinity was found necessary, an excess frequently resulted in a marked reduction in the amount of color produced in the reaction. Folin's carbonate solution was found to be more color-depressing, generally, than Danielson's borax reagent. Ammonium hydroxide and certain aliphatic amines, particularly methylamine, ethylamine, isopropylamine, *d*-glucosamine, etc., were extremely sensitive and the amount of color produced with the quinone reagent decreased rapidly with increased alkalinity. Most of the aromatic amines gave highly colored, insoluble reaction products, but those which could be studied quantitatively did not seem to be greatly influenced by variations in alkalinity.

When the naphthoquinonesulfonic acid reagent was added to a faintly alkaline solution of a compound containing a reactive amino group a deep blackish brown color rapidly developed, especially in the presence of sodium carbonate. Upon acidification the color of the solutions generally became reddish brown. While studying the quinone reaction with certain aliphatic amines, however, the author noticed that the addition of the strong hydrochloric acid bleaching agent used in the Danielson method made the deep blackish brown solutions of certain amines turn such a light yellow, instead of the expected orange-red or reddish brown, that a match with the brown glycine standard was obviously impossible. Upon realkalization the original blackish brown color was regained; hence the colored quinone-amine condensation product had not been destroyed by the acid bleaching agent. The addition of the acetic acid–acetate solution in the Folin method always gave the expected red-brown color. This reagent never produced the light yellow color.

This difference in color production was found to be due to the fact that the Folin reaction solution, after the addition of the various reagents and dilution to 25 cc., had a pH of about 3.2, whereas the corresponding Danielson solution had a pH below 1.2. This difference in hydrogen-ion concentration, in those cases in which the naphthoquinonesulfonic acid–amine condensation product proved to be an indicator compound, was sufficient to alter markedly the color of the resultant solution. Thus *n*-butylamine, *n*-heptylamine, benzylamine, anisidine, aminobenzoic acid, etc., gave insoluble products readily removable by filtration. When these precipitates were dissolved or suspended in alcohol and then added in small quantities to a series of tubes containing buffer solutions ranging from pH 0 to pH 14, many proved to be indicator compounds frequently with two transition intervals. Thus they yielded solutions which were generally yellow from pH 0 to pH 2 and from pH 12 to 14, the intervening color being usually reddish brown or orange-red. Obviously Danielson's method cannot be used to determine the concentration of amines without further modification of the acid bleaching reagent. However, none of the products studied had transition intervals in the region covered by the Folin acetic acid–acetate reagent; hence no error of this type was introduced by this solution.

QUANTITATIVE NATURE OF REACTION BETWEEN NAPHTHOQUINONESULFONIC ACID REAGENT AND AMINES. From a fresh aqueous stock solution of the compound being studied aliquot portions were accurately diluted to volume with distilled water, yielding a series of solutions whose nitrogen content generally ranged from 0.042 to 0.112 mg. per 5 cc. of solution. The previously determined optimal amount of alkali was added to 5-cc. portions and the contents were diluted to 10 cc. and treated with 2 cc. of freshly prepared 0.5 per cent naphthoquinonesulfonic acid reagent. The stoppered tubes were placed in the dark and 24 hours later treated with the desired amounts of the acid and thiosulfate solutions, followed by dilution to 25 cc. The unknown solutions were then matched in the colorimeter against the usual glycine standards which generally contained 0.07 mg. of nitrogen.

TABLE I. QUANTITATIVE NATURE OF THE REACTION BETWEEN VARIOUS AMINES AND SODIUM 1,2-NAPHTHOQUINONE-4-SULFONATE

Compound Studied	Carbonate Solution Added	Folin Colorimetric Method: Mg. of Nitrogen per 5 Cc. of Solution — Nitrogen Recovered: 0.042 mg.	0.056 mg.	0.070 mg.	0.084 mg.	0.112 mg.	Color match with glycine	Danielson Modification of Folin Method: Borax solution added	Acid formaldehyde	Nitrogen recovered
	Cc.	%	%	%	%	%		*Cc.*	*Cc.*	%
Allylamine	0.5	111.2	98.2	100.0	96.5	96.9	Good	0.8	1.0	98.7
o-Aminophenol	0.5	133.3	119.1	125.0	...	125.0	Fair, more orange	1.0	2.0	117.7
m-Aminophenol	0.4	141.2	152.5	154.0	151.6	143.3	Fair, more orange	2.0	2.0	154.0
p-Aminophenol	0.6	208.4	208.5	200.0	208.3	186.5	Fair, more orange	1.0	2.0	200.0
Ammonium hydroxide	0.4	93.3	103.6	95.9	83.3	89.6	Poor, too red	0.8	2.0	101.0
sec-Butylamine	0.6	...	53.2	50.5	46.5	43.3	Fair, too yellow		Acid-sensitive	
Isobutylamine	0.4	87.7	71.8	60.6	59.5	55.5	Poor, too yellow		Acid-sensitive	
Cadaverine dihydrochloride	0.4	115.0	104.1	99.0	92.6	76.2	Fair	0.3	0.8	97.9
Di-*n*-amylamine	0.6	123.6	112.7	102.0	104.3	94.0	Fair, too orange		Acid-sensitive	
Di-*n*-butylamine	0.7	133.3	118.6	101.1	92.6	74.4	Fair, too orange		Acid-sensitive	
Diisobutylamine	1.0	61.7	56.8	52.0	45.0	37.9	Good		Acid-sensitive	
Diethanolamine	0.3	104.3	89.3	83.3	72.5	62.5	Good	0.5	0.1	76.9
Diethylamine	0.7	119.1	104.1	97.6	93.7	72.7	Fair		Acid-sensitive	
Di-*n*-propylamine	0.8	113.8	110.0	106.1	104.2	90.6	Fair, too orange		Acid-sensitive	
Diisopropylamine		No reaction							No reaction	
Ethanolamine	0.6	119.0	109.2	102.9	96.5	91.9	Good	1.0	2.0	100.3
Ethylamine	0.2	54.3	53.2	50.1	46.3	45.6	Good	0.3	0.4	50.8
Ethylenediamine		No reaction							No reaction	
d-Glucosamine hydrochloride	0.1	100.4	95.3	96.4	87.9	78.1	Good	0.2	2.0	96.1
Methylamine	0.2	107.1	105.3	100.0	97.6	96.4	Perfect	0.2	2.0	97.4
Propanolamine	0.2	133.3	131.6	137.0	138.9	138.9	Fair, more orange	0.2	2.0	132.1
n-Propylamine	0.2	...	...	62.6	60.4	56.8	Fair, too yellow	0.5	0.4	59.7
Isopropylamine	0.1	52.1	53.6	47.6	46.3	43.1	Poor, too yellow	1.0	0.7	55.6
Putrescine dihydrochloride	0.5	104.3	100.0	102.0	101.7	105.0	Good		Insoluble	
p-Sulfanilic acid	0.0	185.4	195.5	192.3	136.0	110.0	Fair, more orange	0.0	2.0	192.3
o-Toluidine hydrochloride	1.0	115.0	102.2	103.1	106.9	Ppt.	Good		Insoluble	
Tyramine hydrochloride	0.5	110.5	119.1	111.0	104.2	104.2	Fair, more red	1.5	2.0	99.0

The data on the reaction with 27 different compounds are given in Table I and include the optimal amount of alkali and acid reagents, the per cent of nitrogen recovered, and the color match with glycine. Complete data for the Folin method only are reported. In evaluating the quantitative nature of the reaction one should utilize particularly the data in column 5 and 11 of Table I, where the per cent nitrogen recovery is given when the amine samples and the glycine standards have the same amount of nitrogen—namely, 0.070 mg. This precaution is necessary, since the data indicate that the reaction shows poor proportionality.

While the data show a certain uniformity, in that equimolecular quantities of ammonium hydroxide and 11 of the 26 reactive amines studied gave the same amount of color with the naphthoquinone reagent as an equivalent amount of glycine, certain irregularities are likewise present. Whereas the aliphatic secondary amines, ammonium hydroxide, and certain primary amines such as methylamine, allylamine, and ethanolamine reacted fully with the reagent, ethylamine, *n*-propylamine, *sec*-butylamine, and most of the other primary aliphatic amines yielded only one-half the expected amount of color. Presumably only one of the hydrogen atoms of the amino group of these compounds entered into the reaction. The color given by these derivatives was somewhat more yellow, whereas that given by the secondary amine derivatives was slightly more red or orange than that given by the glycine derivative. Steric hindrance probably accounts for the fact that diisopropylamine and ethylenediamine were completely nonreactive. On the other hand, cadaverine and putrescine resulted in quantitative color production.

In addition, the higher aliphatic amines, such as *n*-butylamine, isoamylamine, *n*-heptylamine, 2-aminoöctane, etc., gave insoluble orange-red precipitates. Tertiary amines of course did not react. Although *p*-aminophenol and *p*-sulfanilic acid gave double the amount of color, when based on an amount of nitrogen equivalent to that present in the glycine standards, most of the aromatic amines studied, such as benzylamine, *o*-, *m*-, and *p*-nitroaniline, anisidine, indole, etc., also gave insoluble reaction products. The Folin method and the Danielson modification tend to give similar results unless the 1,2-naphthoquinonesulfonic acid derivative is sensitive to Danielson's strongly acid formaldehyde solution. Although most of the compounds reacted only in the presence of alkali, certain aromatic amines were found to react with the naphthoquinone reagent equally well in slightly acid solution. This fact enabled the author to develop methods for the determination of sulfanilamide in blood (*13*) and in cerebrospinal fluid (*14*).

The following compounds were completely nonreactive by both the Folin method and the Danielson modification: skatole, guanidine carbonate, hexamethylenetetramine, phenylhydrazine, glycocyamine, melamine hydrochloride, adenine, guanine, xanthine, uracil, hypoxanthine, theobromine, caffeine, phenylurea, urethane, acetanilide, carbanilide, acridine, acetyl anisidine, acetyl phenol, and alloxan.

Summary

The amount of color given by a series of amines which react with sodium 1,2-naphthoquinone-4-sulfonate was found to be influenced by the quantity of alkali and acid added to the reaction medium.

The quantitative nature of the reaction was studied by comparing the amount of color given by ammonium hydroxide and 26 different aliphatic and aromatic amines with that given by an equivalent quantity of glycine.

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Sensitivity of the Carbonate Test for Lithium

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THOUGH lithium carbonate is mentioned as a characteristic slightly soluble lithium compound in comprehensive works on qualitative analysis, no previous investigation appears to have been made of the sensitivity of a qualitative test based upon the precipitation of lithium as the carbonate. From the solubility of lithium carbonate in water at room temperature (1.31 grams per 100 ml. of solution at 20° C. according to Mellor, *1*), one would expect that such a precipitation test would be very insensitive when performed by adding a sodium carbonate solution to a solution of a lithium salt at ordinary temperatures. Experiment showed this to be the case. For example, the addition of 1 ml. of sodium carbonate reagent of any concentration to 1 ml. of lithium chloride solution containing 10 mg. of lithium was found to cause no precipitation at room temperatures. On the other hand, on heating such a mixed solution to 100° C. an abundant precipitation of lithium carbonate resulted, as might be expected from the marked decrease in the solubility of lithium carbonate with rise in temperature (*1*). By performing the test at this elevated temperature considerably less than 10 mg. of lithium can be detected, as is shown in Table I.

TABLE I. PRECIPITATION OF LITHIUM CARBONATE BY SODIUM CARBONATE SOLUTIONS

(From pure lithium chloride solutions at 100° C.)

Lithium Present	Lithium Solution	Reagent Solution	Appearance or Nonappearance of Precipitate with Reagent of Stated Normality		
Mg.	*Ml.*	*Ml.*	*N*	2 *N*	3 *N*
10	1	1	+	+	+
10	1	2	+	+	+
10	2	1	+	+	+
10	2	2	+	+	+
10	2	3	+	+	+
10	1	5	−	−	−
10	5	1	−	−	−
5	1	1	+	+	+
5	1	2	−	+	−
5	1	3	−	−	−
4	1	1	−	+	+
4	1	2	−	−	−
3	1	1	−	+	−
3	1	2	−	−	−
2	1	1	−	−	−
2	1	2	−	−	−

Table I shows clearly the need for closely restricting the volumes of the reacting solutions. A certain optimum concentration of sodium carbonate is required for best results. That potassium carbonate is a slightly less sensitive reagent than sodium carbonate in solutions of equivalent concentration is shown by Table II.

TABLE II. PRECIPITATION OF LITHIUM CARBONATE BY 2 *N* POTASSIUM CARBONATE

(From pure lithium chloride solutions at 100° C.)

Lithium Present	Lithium Solution	Reagent Solution	Appearance or Nonappearance of Precipitate
Mg.	*Ml.*	*Ml.*	
10	1	1	+
10	1	2	+
10	2	1	+
10	2	2	+
10	2	3	+
10	1	5	−
10	5	1	−
5	1	1	+
5	1	2	−
4	1	1	+
4	1	2	−
3	1	1	−
3	1	2	−

Table III shows the influence of various concentrations of sodium or potassium ion on the sensitivity of this test.

In each of these experiments the volume of the test solution was 1 ml., and 1 ml. of reagent was used. It will be seen that a high concentration of sodium ion has very little adverse effect on the precipitation of lithium carbonate, whereas potassium ion in high concentration has a slightly more noticeable effect. However, neither sodium nor potassium interferes very much with this test for lithium. For the detection of lithium in the presence of other alkalies the following procedure is satisfactory:

Reduce the solution to be tested to a volume of about 1 ml., transfer to a small test tube, and add 1 ml. of 2 *N* sodium carbonate solution. After mixing the solutions well, stopper the test tube loosely and place it in boiling water for about 10 minutes. The presence of lithium is shown by the appearance of a white crystalline precipitate which usually adheres to the side of the test tube. Care must be taken not to prolong the test to such an extent that salts separate from the solution from evaporation.

TABLE III. PRECIPITATION OF LITHIUM CARBONATE WITH 2 *N* SODIUM OR POTASSIUM CARBONATE

(From lithium chloride solutions containing added sodium or potassium chloride)

Li Present	Na Added	K Added	Reaction with Given Reagent	
			Na_2CO_3	K_2CO_3
Mg.	*Mg.*	*Mg.*		
4	25	0	+	+
4	50	0	+	+
4	100	0	+	+
4	0	25	+	+
4	0	50	+	+
4	0	100	+	+
3	25	0	+	−
3	50	0	+	−
3	100	0	+	−
3	0	25	+	−
3	0	50	+	−
3	0	100	−	−

Though 3 mg. is the smallest amount of lithium that can be detected by this particular procedure, correspondingly smaller amounts can be detected by reduction of the volumes of the reacting solutions. However, in working with very small volumes a special technique must be employed to avoid error caused by evaporation. A convenient microchemical modification of the test is the following:

By means of a capillary pipet place one or two drops of the unknown solution in the bottom of a short length of 6-mm. glass tubing which has been sealed at one end. In a similar way introduce one or two drops of 2 *N* sodium carbonate solution and mix the solutions by means of a fine platinum wire. Seal off the open end of the tube as close to the liquid as possible. Place the prepared capsule in an ordinary test tube containing distilled water, heat to boiling, and maintain at the boiling point for at least 5 minutes. In the presence of a few tenths of a milligram of lithium a white crystalline precipitate will separate on the walls of the capsule.

Interfering Substances

Ammonium salts increase the solubility of lithium carbonate to a marked extent and should therefore be removed from the solution before applying the test. Of course nearly all other cations give a precipitate with carbonate ion and thus interfere with the immediate application of the test. However, by taking advantage of the fact that lithium carbonate is the only metal carbonate which exhibits marked retrograde solubility with rise in temperature, the test may be applied after removal of other metal ions as carbonates by precipitation in cold solution. It is advisable to precipitate the interfering cations in dilute solution at around 0° C. with just a sufficient amount of dilute sodium carbonate solution. After removal of the precipitated carbonates by filtration, the solution is then concentrated by vacuum evaporation at room temperature or below. A second filtration to remove separated salts may be necessary before the lithium test can be applied to the small volume of liquid that must be used.

Conclusions

Though the carbonate reaction for lithium is too insensitive at room temperature to be of much practical use, the increase in sensitivity when the test is performed at 100° C. is such that the reaction becomes as sensitive and useful as some other qualitative reactions. About 3 mg. is the least amount that can be detected by a macromethod, but by the application of microchemical technique a few tenths of a milligram can be detected.

The other alkalies do not interfere with the test. Ammonium salts prevent precipitation and must be removed. Nearly all other interfering cations may be conveniently removed by precipitation as carbonates in cold solution, the test then being applied to the filtrate after concentrating it to the proper volume. The carbonate test for lithium is the most nearly specific of the known precipitation reactions for lithium, though it is not nearly so sensitve as tests based upon precipitation as aluminate, arsenate, fluoride, phosphate, stearate, or triple uranyl acetate. In spite of its comparatively low sensitivity it may be useful for establishing the presence of lithium as an essential constituent of an unknown material when a satisfactory decision as to the approximate amount present cannot be obtained by the usual flame or spectroscopic tests.

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Determination of Carbonyl Compounds by Means of 2,4-Dinitrophenylhydrazine

Water-Insoluble Carbonyl Compounds

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THE use of 2,4-dinitrophenylhydrazine in the qualitative identification of carbonyl compounds has been developed extensively by Allen (*1*), Brady (*2*), and Campbell (*3*), and its use as a quantitative reagent has been reported frequently for individual carbonyl compounds (*4*, *6*, *7*) and for a group of water-soluble carbonyl compounds by Iddles and Jackson (*5*).

Since many carbonyl compounds are insoluble or only slightly soluble in water, it seemed desirable to extend the earlier study (*5*) to include other carbonyl compounds which may be dissolved in alcohol. Consequently the present report is concerned with a study of the best conditions for the quantitative precipitation of certain alcohol-soluble carbonyl compounds as their 2,4-dinitrophenylhydrazones.

Experimental

In adapting the previous work in aqueous solutions to carbonyl compounds soluble in alcohol, preliminary trials were run on a representative alcohol-soluble ketone, acetophenone, to determine (1) the effect of temperature on the completeness of reaction and (2) the final dilution necessary to ensure a quantitative precipitation of the hydrazone.

In the determination of carbonyls reported from this laboratory, the temperature was held at 0° C. This led to the suggestion by Perkins and Edwards (*6*) that some occlusion of the reagent might occur when the reagent was saturated at room temperature but was used in a reaction which was cooled down to 0° C. To test this point three parallel trials were made as shown in Table I, in one of which the reagent was saturated at 0° C. and the run made at the same temperature, and in the others the reagent was saturated at room temperature and the runs were made at 0° C. and at room temperature.

The close agreement of the results shown in Table I indicates that the reagent was used up by the reaction sufficiently to compensate for any decrease in its solubility when a lower temperature was employed. In another test 50 ml. of precipitating reagent (saturated at room temperature) and 10 ml. of water, the minimum volume of carbonyl solution previously used, were mixed and cooled to 0° C. No precipitate was produced, showing that the dilution effect was sufficient to prevent precipitation of the reagent and any resulting occlusion. From these data it was concluded that determinations could be made at 0° C. or at room temperature. However, room temperature was selected for all later runs, as it offered the advantages of better particle size of precipitates, greater ease of filtration, and sufficiently low solubility of the hydrazones formed.

In determining the effect of dilution upon the precipitation of the hydrazones, trials were made in which 0, 50, and 100 ml. of 2 *N* hydrochloric acid were added after precipitation.

TABLE 1. DETERMINATION OF ACETOPHENONE

	Volume of 2,4-Dinitrophenylhydrazine	Volume of Sample	Sample	Precipitation
	Ml.	*Ml.*	*Gram/ml.*	%
Reagent saturated at 0° C. Determination made at 0° C.	30	10	0.006072	99.1
	30	10		99.8
	30	10	0.00647	99.9
	30	10		99.3
Reagent saturated at room temperature. Determination made at 0° C.	30	10	0.006072	99.9
	30	10		99.7
	30	10	0.00647	99.9
	30	10		99.1
Reagent saturated at room temperature. Determination made at room temperature	30	10	0.00647	99.4
	30	10		99.75

TABLE II. EFFECT OF DILUTION IN THE DETERMINATION OF ACETOPHENONE

Volume of 2,4-Dinitrophenylhydrazine	Volume of Carbonyl[a]	Volume of 2 *N* Hydrochloric Acid	Total Volume	Precipitation
Ml.	*Ml.*	*Ml.*	*Ml.*	%
30	10	...	40	99.0
30	10	...	40	98.6
30	10	50	90	99.1
30	10	50	90	98.1
30	10	100	140	98.8
30	10	100	140	98.6

[a] 0.0647 gram per 10 ml.

TABLE III. DETERMINATION OF CARBONYL COMPOUNDS AS 2,4-DINITROPHENYLHYDRAZONES

	Volume of Hydrazine *Ml.*	Volume of Carbonyl *Ml.*	Sample *Gram/ml.*	Weight of Hydrazone *Gram*	Precipitation %
Acetophenone (b. p. 202° C.)	30	10	0.006072	0.1517	99.9
	30	10		0.1515	99.8
	30	10		0.1520	100.05
	30	10		0.1520	100.05
	30	10		0.1500	98.9
	30	10		0.1502	99.2
	30	10		0.1505	99.3
	30	10		0.1512	99.7
	30	10	0.00647	0.1618	100.05
	30	10		0.1615	99.9
	30	10		0.1601	99.1
	30	10		0.1604	99.3
	30	10		0.1611	99.7
	30	10		0.1598	98.7
	30	10	0.006246	0.1554	100.0
	30	10		0.1556	100.0
	30	10		0.1554	100.0
	30	10		0.1555	100.0
	30	10		0.1554	100.0
				Av.	99.6
p-Hydroxybenzaldehyde (m. p. 116–117°)	30	10	0.006	0.1521	96.7
	30	10		0.1523	96.9
	30	10		0.1514	96.2
At room temperature	30	10		0.1516	96.3
	30	10		0.1530	97.2
At 0°	25	10	0.005324	0.1313	99.8
	25	10		0.1312	99.75
				Av.	97.6
Benzoin (m. p. 137° C.)[a,b]	25	10	0.002885	0.0537	100.80
	25	10		0.0539	100.95
	25	10		0.0537	100.80
	25	10		0.0532	99.75
	25	10	0.003719	0.0681	99.0
	25	10		0.0678	98.7
	15	10		0.0666	97.0
	15	10		0.0686	99.8
				Av.	99.6
Mesityl oxide (b. p. 130° C.)	35	10	0.006503	0.1687	91.5
	35	10		0.1691	91.8
	35	10		0.1725	93.5
	35	10		0.1731	93.9
	35	10	0.006108	0.1604	92.2
Mesityl oxide (b. p. 130° C.)	35	10		0.1624	93.9
	35	10		0.1640	94.8
	28	10		0.1620	93.7
				Av.	93.2
Benzalacetophenone (m. p. 55.1° C.)	10	10	0.002028	0.0360	96.8
	10	10		0.0368	99.0
	15	10		0.0374	100.6
	7.5	10		0.0362	97.45
				Av.	98.5
Benzil (m. p. 95° C.)[c]	40	10	0.007826	0.1413	97.5
	40	10		0.1416	97.6
	40	10		0.1421	98.0
				Av.	97.7
Benzophenone (m. p. 47–48° C.)	18.5	10	0.004218	0.0807	96.2
	23	10		0.0807	96.2
	23	10		0.0782	94.5
				Av.	95.6
Piperonal (m. p. 37° C.)[d]	55	10	0.013837	0.3128	102.6
	75	10		0.3156	103.2
	75	10		0.3184	104.3
	55	10		0.3166	103.8
				Av.	103.5
Cyclohexanone (Eastman) redistilled four times	80	10	0.01	0.2761	97.4
	80	10		0.2748	96.9
	80	10		0.2753	97.1
	80	10		0.2751	97.0
	80	10		0.2762	97.4
	80	10		0.2765	97.50
	80	10		0.2769	97.63
				Av.	97.3
Cyclopentanone (Eastman) redistilled four times	53	10	0.007537	0.2316	98.0
	53	10		0.2324	98.5
	53	10		0.2318	98.0
	71	10		0.2331	98.6
	71	10		0.2324	98.5
	92	10	0.01	0.3141	99.95
	92	10		0.3096	98.54
				Av.	98.6
Carvone (Eastman) redistilled four times	56	10	0.01	0.2072	98.76
	56	10		0.2084	99.33
	42	10		0.2091	99.71
	42	10		0.2084	99.34
	42	10		0.2093	99.76
				Av.	99.38

[a] Reaction incomplete at 0° C.
[b] Only a hydrazone, as shown by Rabassa (*7*).
[c] Calcd. on basis of monohydrazone.
[d] Large crystals of hydrazone with occlusion.

As shown in Table II, there is no apparent effect produced by further decreasing the concentration of alcohol beyond the dilution caused by the aqueous reagent itself.

From all the experimental trials on acetophenone the optimum conditions selected for a general procedure were as follows:

A known weight of the purified carbonyl compound was diluted to 100 ml. with aldehyde- and ketone-free 95 per cent ethyl alcohol. Ten-milliliter aliquot portions of this stock solution were added dropwise, with continuous stirring, to a volume of the 2,4-dinitrophenylhydrazine reagent (a saturated solution in 2 *N* hydrochloric acid) equivalent to 50 to 100 per cent excess of that theoretically required for complete precipitation. The precipated solutions were usually diluted by addition of 50 ml. of 2 *N* hydrochloric acid and allowed to digest at room temperature for 2 to 24 hours. The precipitates were then filtered onto tared Gooch crucibles, washed with 100 to 150 ml. of 2 *N* hydrochloric acid, then with distilled water until the washings gave no test with silver nitrate, and dried in an oven at 105° to 110° C. to constant weight.

This procedure was used to study the completeness of precipitation of the 2,4-dinitrophenylhydrazones of acetophenone, *p*-hydroxybenzaldehyde, benzoin, mesityl oxide, benzalacetophenone, benzil, benzophenone, piperonal, cyclohexanone, cyclopentanone, and carvone. The results are tabulated in Table III and show a very satisfactory efficiency of recovery for organic quantitative precipitations.

Conclusions

Supplementing an earlier study (*5*) on water-soluble carbonyl compounds, the authors have sought to determine the optimum conditions for the quantitative estimation of alcohol-soluble carbonyl compounds as their 2,4-dinitrophenylhydrazones. In the experimental determinations a sample containing a small quantity of the carbonyl compound in alcoholic solution was added to an excess of 2,4-dinitrophenylhydrazine in 2 *N* hydrochloric acid and the reaction mixture was allowed to stand at room temperature after dilution with 50 ml. of 2 *N* hydrochloric acid. The precipitate was filtered, washed with 2 *N* hydrochloric acid and water, and dried at 105° to 110° C. to a constant weight.

Determinations of the amounts of hydrazone produced from samples of eleven aldehydes and ketones were made and compared with the known theoretical values with variations of −0.4 per cent for acetophenone, −2.4 per cent for *p*-hydroxybenzaldehyde, −0.4 per cent for benzoin, −6.8 per cent for mesityl oxide, −1.5 per cent for benzalacetophenone, −2.3 per cent for benzil, −4.4 per cent for benzophenone, +3.5 per cent for piperonal, −2.7 per cent for cyclohexanone, −1.4 per cent for cyclopentanone, and −0.62 per cent for carvone.

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Determination of Beta-Carotene in Alfalfa Meals

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THE importance of alfalfa meals as a source of carotene for commercial feed mixtures has necessitated the development of an analytical technique suitable for routine analysis. Four methods useful for this purpose have been studied (*1*, *2*, *4*, *5*) and from experience with these a comparatively rapid and satisfactory procedure has been developed. Five outstanding advantages distinctly in favor of this technique are: (1) 125-cc. separatory funnels are employed, and proportionately small amounts of solvents and reagents used; (2) twelve or more determinations may be run simultaneously; (3) ten to twelve water washings are eliminated; (4) concentration of the petroleum ether extract is unnecessary; and (5) the photelometer is used in place of a colorimeter.

A special type of shaker (Figure 1), which has been developed in this laboratory, for the determination of carotene in alfalfa meals, greatly facilitates the many extractions and washings required.

The shaking apparatus consists essentially of a shaker rack and a shaker carriage geared at right angles to a 0.16-horsepower General Electric motor (Figure 2). The shaker rack is hinged to the carriage in such a way that it may be brought to an upright position after each shaking operation (Figure 3). The shaker rack is constructed from 1.25-cm. (0.5-inch) stock braced with 0.6-cm. (0.125-inch) plywood; 125-cc. separatory funnels rest in rubber collars in holes having a diameter of 3.75 cm. (1.5 inches). During the process of shaking, the glass stoppers of the separatory funnels are securely held in place by a hinged toppiece, the wing of each stopper passing through a slot in the

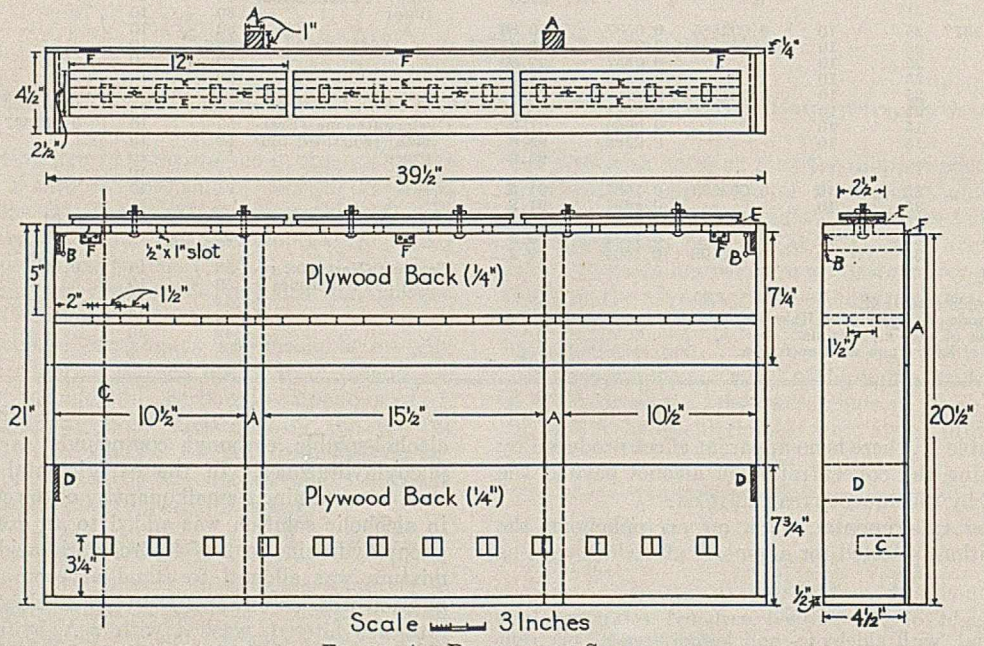

FIGURE 1. DIAGRAM OF SHAKER

A. Cleats for shaker carriage
B. Hook fastener
C. Clamp for flask
D. Cleat for funnel rack (Figure 4)
E. Rubber tubing (1.25-cm.)
F. Hinge

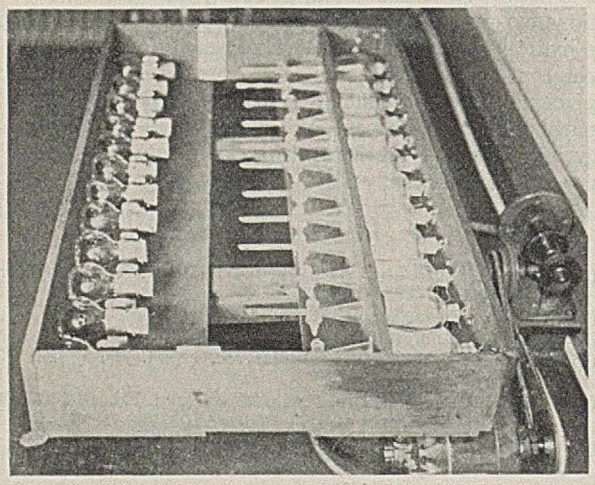

FIGURE 2. SHAKER AND MOTOR

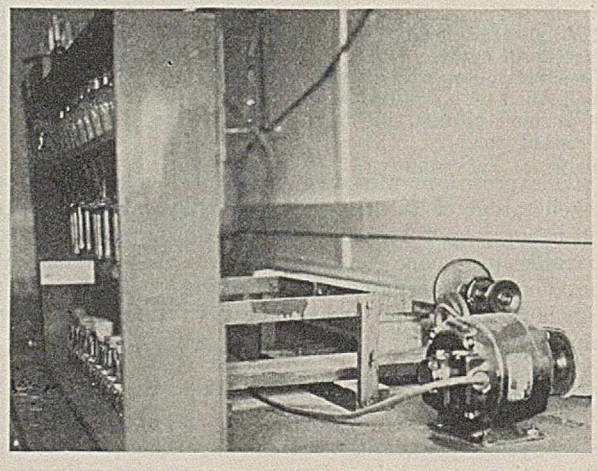

FIGURE 3. MOTOR AND UPRIGHT SHAKER

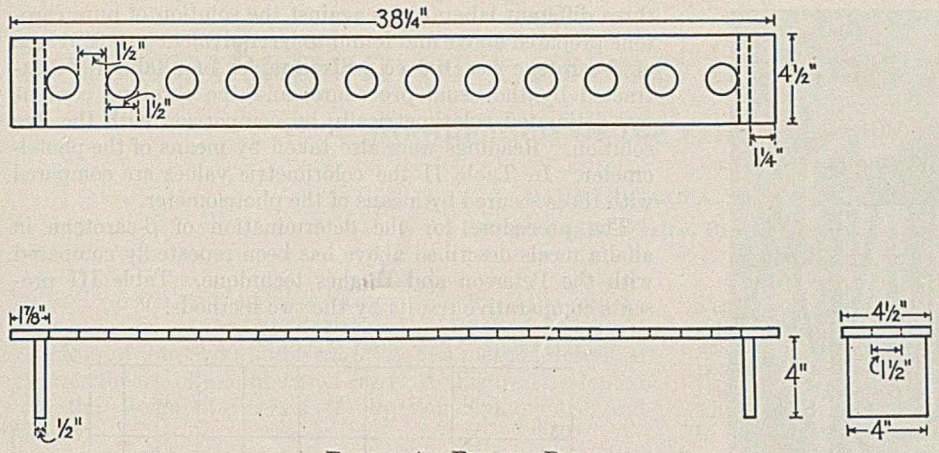

FIGURE 4. FUNNEL RACK

toppiece. Strips of plywood, held in place by bolts and adjustable by wing nuts, secure the stoppers.

Lengths of 1.25-cm. (0.5-inch) rubber tubing attached to the under side of the plywood strips facilitate adjustment. Very little adjusting is necessary once the apparatus is assembled. Phosphor bronze clamps are used to hold 100-cc. extraction flasks in place. A rack for holding the glass funnels, used in transferring the petroleum ether extract into the separatory funnels, is a great convenience (Figures 4 and 5), and may also be used in the final filtration of the carotene solution into volumetric flasks (Figure 5, lower).

Determination

Weigh 2 grams of finely ground alfalfa meal into a 100-cc. fat-extraction flask, add 15 cc. of 10 per cent solution of potassium hydroxide in 95 per cent ethanol, and wash down the sides of the flask with about 5 cc. of 95 per cent ethanol. It is best to filter the alcoholic solution before use. Attach the flask to a reflux condenser and boil the contents (Figure 6) for 30 minutes. It is advisable to lift and rotate the flask occasionally to keep the sample from lumping. Remove the flask and cool quickly to room temperature.

Add 15 cc. of petroleum ether to the flask and cork tightly. Place in a clamp on the shaker (Figure 5, upper), lower the shaker rack onto the carriage, and shake for 2 minutes. Rotate flask occasionally by hand while shaking. Elevate the shaker to the perpendicular position and filter the supernatant liquid into the separatory funnel through a short-necked glass funnel (6.25 cm., 2.5 inches, in diameter) containing a small loose plug of nonabsorbent cotton (Figure 5, center). Grease and secure the stopper of the separatory funnel. The stoppers of the separatory funnels must be frequently greased during the whole procedure. After three extractions, add 10 cc. of 95 per cent ethanol to break up the residue, and then extract with 15-cc. portions of petroleum ether as above until the extractions are colorless. Six extractions in all usually suffice, although this will vary according to the sample.

Remove the filter rack, fill the separatory funnel with distilled water, and allow to stand for 15 minutes. Slowly drain off the water layer almost completely, leaving about 1 cc. to act as a seal.

Add a 20-cc. portion of distilled water to the separatory funnel, secure the stopper as described above, lower the shaker rack onto the carriage, and shake for 2 minutes. Elevate the shaker rack and run off the water layer. After this single washing with water, wash the solution repeatedly with 20-cc. portions of 89 per cent methanol until the methanol remains water-white and is found to be free of alkali. Finally filter the petroleum ether extract directly into 100-cc. volumetric flasks through S. & S. No. 597 11-cm. paper on which has been placed 1 gram of anhydrous sodium sulfate, and make the solution up to volume, usually 100 cc., with petroleum ether (Figure 5, lower). Determine the concentration of β-carotene by means of a photelometer (*6*).

For best results the shaker should operate at 60 to 65 oscillations a minute. The methanol is readily recoverable, and it has been the authors' experience that the redistilled solvent is preferable to the original methanol. Very little emulsification is encountered when the recovered alcohol is used.

ALTERNATIVE METHOD OF EXTRACTION (*3*). After digestion for 0.5 hour cool the contents of the flask and then pour into a sintered-glass funnel (No. 15180 A, Jena glass, with fritted-glass disks fused in place, Cenco catalog) which is attached to a 0.5-liter suction flask. Apply suction until most of the solvent is removed. Wash the residue on the plate alternately with 25-cc. portions of petroleum ether and absolute alcohol until the filtrate comes through clear. The suction should at no time be applied unless the sediment is partially covered with solvent. After the addition of each wash portion of solvent, more complete extraction may be obtained by stirring the sediment and solvent on the funnel plate with a stirring rod before applying suction.

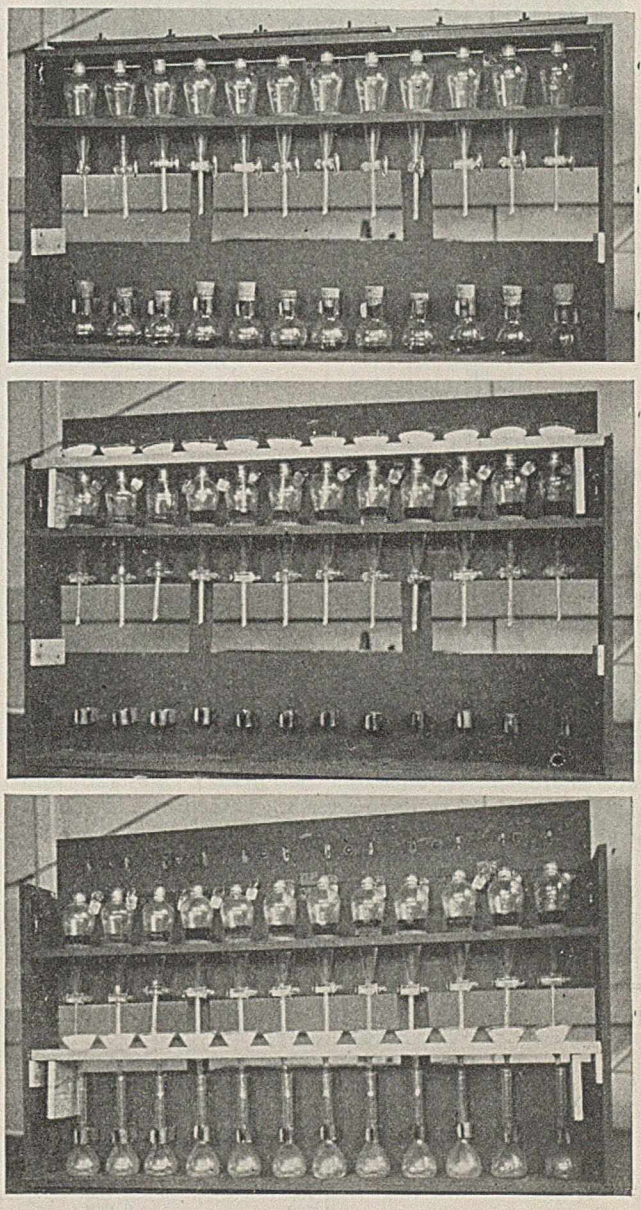

FIGURE 5. RACK WITH FUNNELS AND FLASKS

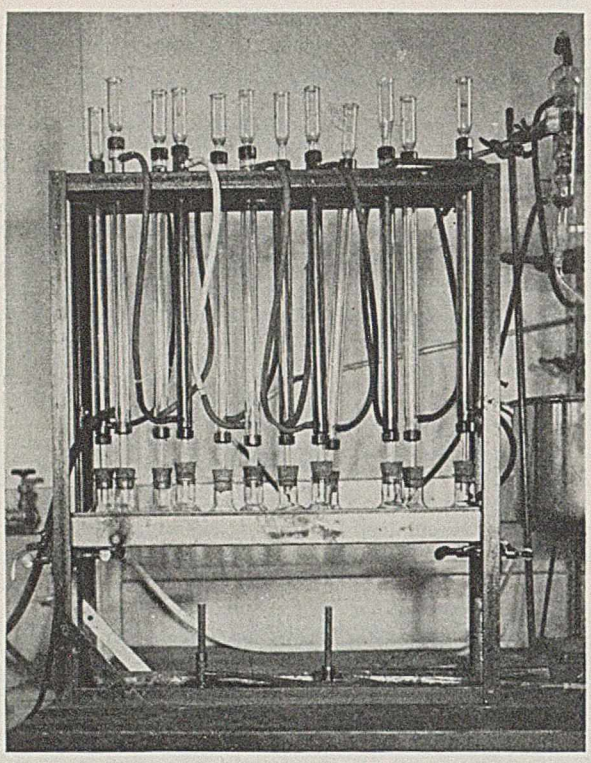

FIGURE 6. FLASKS AND REFLUX CONDENSER

TABLE I. CALIBRATION OF PHOTELOMETER FOR β-CAROTENE DETERMINATION

Concentrations *Mg./100 cc.*	Reading
0.306	28.0
0.153	51.2
0.102	63.5
0.061	75.8
0.041	83.9

To apply the photelometer to the authors' purpose, a combination of two filters—Cenco lantern blue No. 554, and Noviol A (No. 0.038, 2.95 mm., Corning Glass Works)—was used. This combination cuts out all wave lengths below 400 mμ and transmits a maximum of 450 mμ.

The instrument which the authors used in their work was calibrated, using the two filters, by dissolving pure β-carotene crystals in redistilled petroleum ether that had been filtered through anhydrous sodium sulfate to remove traces of moisture, and making the solution up to 1,000 cc. Each cubic centimeter contained 0.0102 mg. of β-carotene. Suitable dilutions were made and readings taken with the photelometer. Table I gives the results.

By plotting the logarithm of the reading against the concentration, a calibration curve was obtained (Figure 7). The curve was then checked colorimetrically, using a dye solution as described by Guilbert (*2*). The dye was standardized in three different laboratories against the solution of pure carotene prepared above and found to be equivalent to 0.3191 mg. of β-carotene per 100 cc. Six samples of alfalfa were extracted by the usual procedure, and the carotene content was estimated colorimetrically by comparison with the dye solution. Readings were also taken by means of the photelometer. In Table II the colorimetric values are compared with those secured by means of the photelometer.

TABLE II. COMPARISON OF RESULTS BY PHOTELOMETER AND COLORIMETER

Sample	β-Carotene	
	Colorimeter	Photelometer
	Mg./100 cc.	
1	0.210	0.210
2	0.130	0.132
3	0.260	0.256
4	0.380	0.365
5	0.100	0.108
6	0.060	0.062

The procedure for the determination of β-carotene in alfalfa meals described above has been repeatedly compared with the Peterson and Hughes technique. Table III presents comparative results by the two methods.

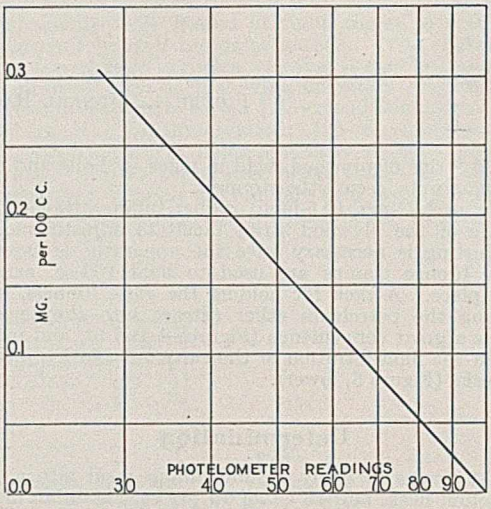

FIGURE 7. CALIBRATION CURVE FOR β-CAROTENE

TABLE III. COMPARISON OF METHODS

Sample	Peterson and Hughes *Mg./100 g.*	Wirthmore *Mg./100 g.*
616	12.76	12.76
276	8.51	8.51
305	11.98	12.01
255	7.52	7.65
230	1.64	1.70
175	1.25	1.26
183	9.26	9.29
92	6.76	6.76
3472	24.61	24.12
3473	8.56	8.67

Summary

The Peterson and Hughes procedure for the determination of β-carotene in alfalfa products has been modified and adapted to the purpose of routine analysis. A convenient shaker greatly facilitates the process of extraction and purification.

The photelometer has been found to be a practical and sufficiently accurate instrument for determining the concentration of β-carotene in petroleum ether extracts from alfalfa meal.

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Determination of Carbon in Organic Compounds

Modification of the Combustion Vessel

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THE carbon content of a variety of nonvolatile organic compounds can readily be determined by the manometric technique of Van Slyke and Neill (*2*). The method described involves the use of a combustion vessel, *A* (Figure 1), adapted from the design of Backlin (*1*) by Van Slyke, Page, and Kirk (*3*).

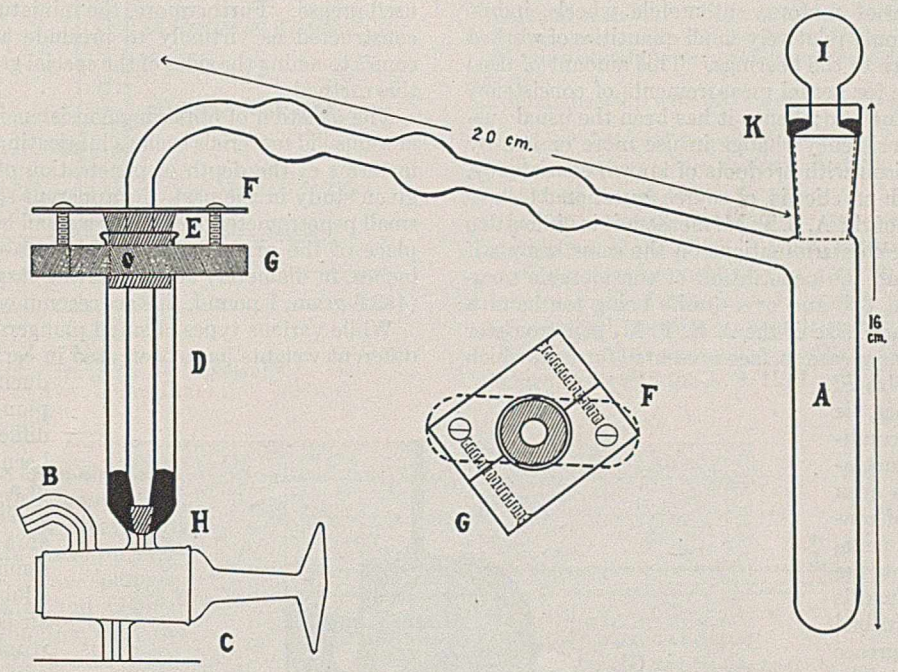

FIGURE 1. MODIFIED COMBUSTION VESSEL FOR CARBON DETERMINATION
Details of Bakelite attachment, *G*, to cup are shown in insert, top view

Carbon dioxide–free sodium hydroxide (0.5 *M*) is admitted into the extraction chamber, *C*, by means of a soda-lime protected separatory funnel at the cup, *D*. Upon removal of the separatory funnel the combustion vessel, *A*, is fitted to the cup as described below. The carbon dioxide liberated by combustion is absorbed by the sodium hydroxide, after which gases other than carbon dioxide are removed under mercury through the side arm, *B*. The combustion vessel is replaced by a buret and a measured quantity of lactic acid is admitted to the extraction chamber. Carbon dioxide is freed and the pressure at a known volume measured. The carbon dioxide is reabsorbed by strong sodium hydroxide and the pressure measured at the same volume. The pressure of carbon dioxide is secured by the difference between the two readings. A detailed description is given by Van Slyke, Page, and Kirk (*3*).

Van Slyke *et al.* (*3*) attach the combustion vessel to *B* by rubber tubing. With this technique accurate analyses may be obtained, but leaks occur around the rubber tubing with sufficient frequency to invalidate many of the results, and the authors have found it more satisfactory to attach the combustion vessel to the extraction chamber through *D*. This necessitated a slight change in the design of the arm of the combustion vessel as pictured in Figure 1. In addition, the arm of the combustion vessel is held rigidly in place by means of a rubber stopper, *E*, through which it passes, and which fits snugly into the top of *D* and is kept in position by means of an aluminum plate, *F*. Screws fasten *F* to a Bakelite plate, *G*, attached to the cup of the extraction chamber. Thus the combustion vessel may be easily and rigidly attached to the extraction chamber.

A rubber tip, *H*, covered by a layer of mercury, prevents gas leaks around the tip of the arm of the combustion vessel. By screwing down the aluminum plate this rubber tip is seated securely in place. As a further precaution the stopper, *I*, of the combustion vessel is sealed with mercury, *K*.

The principal advantage of this new design lies in the fact that it removes all possibility of leaks at the point of union between the combustion vessel and the extraction chamber. The method of analysis is identical with that of Van Slyke, Page, and Kirk (*3*) except that the unabsorbed gas in the extraction chamber is ejected through *B* under mercury instead of through *D*.

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Miniature Penetrometer for Determining the Consistency of Lubricating Greases

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THE accepted method for determining consistencies of lubricating greases is the A. S. T. M. method (*1*), which, however, requires that a considerable quantity of grease be available—i. e., at least 400 to 500 grams for grease of soft or moderately soft consistencies. Such large quantities of grease are readily obtainable in the manufacturing plants or in storage; however, it frequently happens that information on the consistencies of very small samples of grease is desired. For example, after use on ball or roller bearings, such as the antifriction bearings of motors, automobile wheels, industrial machines, etc., only relatively small quantities of worked or used grease adhere to the bearings. This amount of used grease is inadequate for actual measurements of consistency by the A. S. T. M. method; hence it has been the usual custom to estimate consistency change in use more or less by guess or by comparison with products of known consistency. The accuracy of such practice is, of course, questionable.

Again, in the case of the A. S. T. M. method, it is difficult to obtain accurate check determinations on the same sample if the grease is soft and the penetration of considerable magnitude—for example, 300 and over (units being tenths of a millimeter). The large size of the A. S. T. M. penetrometer cone in relation to the grease surface presented for test, which is limited by the size of the grease container, is responsible for this condition. In taking penetrations of soft greases even in the required size of container, the depth of penetration is such that the original grease surface is considerably disturbed and the bulk of the grease slightly worked. The limited area exposed prevents subsequent determinations on undisturbed surfaces, owing to the danger of the cone's touching the sides of the grease container. The only remaining alternative, therefore, is to smooth out the disarranged surface and again determine the consistency in essentially the same spot. Readings increasingly higher than the original value, since repeated working induces softening, are usually obtained as a result of this procedure.

To circumvent the above objections to the A. S. T. M. method a miniature penetrometer has been designed which may be utilized as an adjunct to the A. S. T. M. penetrometer and which permits accurate consistency measurements on small samples of greases—that is, about 3 to 5 grams. With a slight modification of the design, consistencies of even smaller samples could be determined. Generally, more than this quantity of used grease can be readily recovered from antifriction bearings of average size. The miniature penetrometer not only permits positive measurements but also allows check determinations with a small supply of sample, and fairly consistent check results are possible on the same sample of used grease. Furthermore, the miniature penetrometer is so constructed as virtually to preclude any possibility of the cone's touching the sides of the special grease container used in this method.

The question of obtaining consistencies of small quantities of semisolid materials such as lubricating greases, as expressed in terms of the depth of penetration of a plunger, has been given study in the past, the apparent solution being to use a small penetrometer needle and a small holder for the grease in place of the present A. S. T. M. grease cone (6.5-cm., 2.56 inches, in diameter) and the rather large-sized grease holder (453.6-gram, 1-pound, tins are recommended).

While various types of small plungers such as glass rods of different weights have been used in earlier work, thereby reducing the size of the plunger cone, the chief difficulty encountered has been in a suitable design of a small holder for the grease, since charging of a small cylinder with a semisolid material such as a lubricating grease causes air entrainment as well as working down of the grease structure and consequent alteration in consistency, usually softening. Both difficulties are overcome by using a split cylinder for the holder. Each half of the cylinder can be charged with grease by simply using a spatula. In this manner, working down of the grease is practically negligible and air entrainment is reduced to a minimum.

With regard to the penetrometer cone, in the A. S. T. M. method the cone, as stated above, has a maximum radius of 6.5 cm. (2.56 inches), the total weight of cone and plunger being 150 grams. In order to take penetrations of small samples it was necessary to reduce the size and weight of the plunger, which was ac-

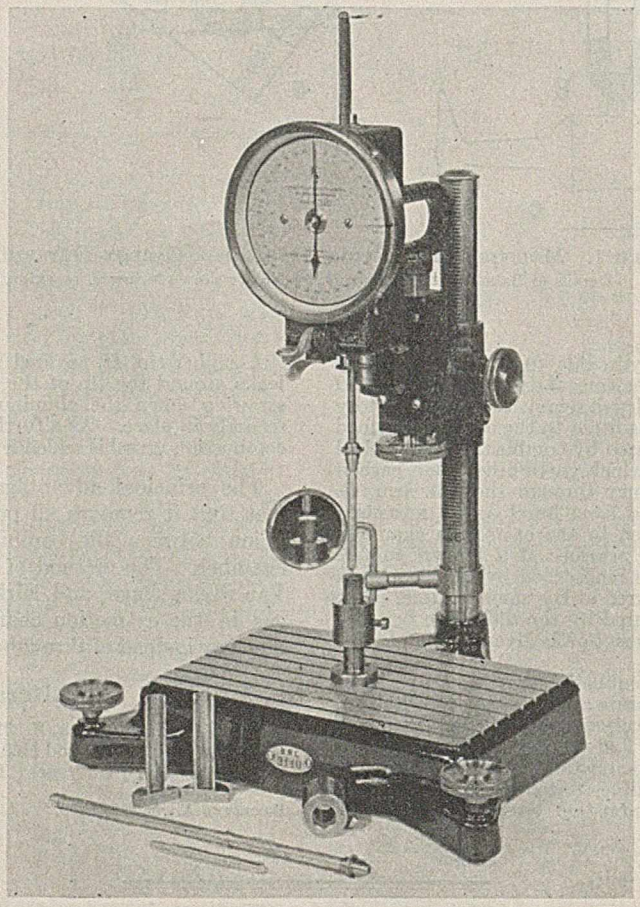

FIGURE 1. MINIATURE PENETROMETER ASSEMBLY

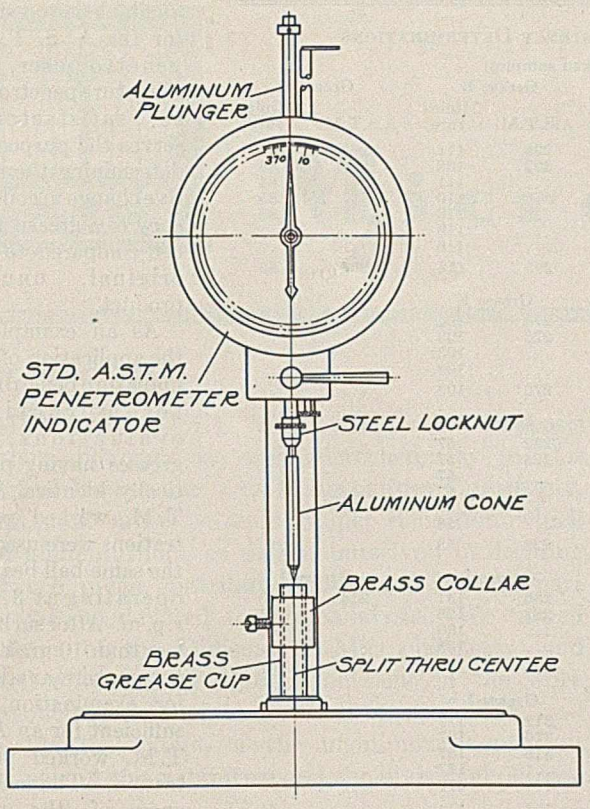

FIGURE 2. DETAILS OF ASSEMBLY

complished by the use of an aluminum plunger and cone with a total weight of but 20 grams. For very hard greases, provisions are made for adding weights to the grease plunger. The small grease holder and plunger are then used in conjunction with the present A. S. T. M. grease penetrometer indicator, thereby reducing the cost of the miniature penetrometer to a minimum.

Description of Apparatus

In Figure 1 is shown a photograph of the miniature penetrometer assembled, and in the foreground are shown the individual parts of the grease holder, plunger, and cone. Figures 2 and 3 give details of construction of the grease cup or holder, aluminum plunger, and aluminum cone.

The grease cup (capacity 4 grams of grease) consists of a split brass bushing 5.715 × 0.952 cm. (2.25 × 0.375 inches) in inside diameter affixed to a suitable base. The penetrometer needle or plunger consists of an aluminum cone which fits into an aluminum plunger and is connected to the A. S. T. M. penetrometer indicator. The total weight of the aluminum cone, plunger, etc., is 20 grams.

The method of obtaining penetrations follows that outlined in A. S. T. M. Designation D217-33T (*1*). After bringing the grease to the usual temperature, 25° ± 0.556° C. (77 ± 1° F.), it is transferred into the small grease holder by means of a spatula, filling each half of the split cylinder. The two halves are then clamped together by means of the brass collar as indicated in Figure 1 and the surface of the grease is smoothed off. In order to ensure centering of the grease holder with the plunger, a centering plate (not shown), with suitable recess to fit the base of the miniature grease holder, is affixed to the base of the A. S. T. M. penetrometer and the grease holder is placed in the recess. This prevents movement of the holder during taking of penetrations. Penetrations are obtained as in the A. S. T. M. method. After each penetration, if check results are desired, additional grease is added to the grease cup, the surface is smoothed off, and the test is repeated. For very hard greases weights can be added to the plunger.

Experimental Data

In order to determine what relationship, if any, exists between this miniature penetrometer and the A. S. T. M. penetrometer, comparative data were obtained on the most common types of greases of different consistencies, as follows:

Calcium soap with oil of low and of high viscosity.
Sodium soap with oil of low and of high viscosity.
Mixture of sodium and calcium soaps with oil of low and of high viscosity.
Aluminum soap with oil of low and of high viscosity.

Results obtained are given in Table I. The range of consistencies of the greases shown is that commonly termed in the trade from No. 00 to No. 3, and penetrations have been compared on the worked sample as specified under A. S. T. M. Designation D217-33T (*1*). As regards reproducibility of results, except for very fibrous greases the miniature penetrometer method compares favorably with the A. S. T. M. method which permits a mean deviation of 3 per cent.

With regard to a possible correlation between the miniature penetrometer and the A. S. T. M. penetrometer, it is seen from

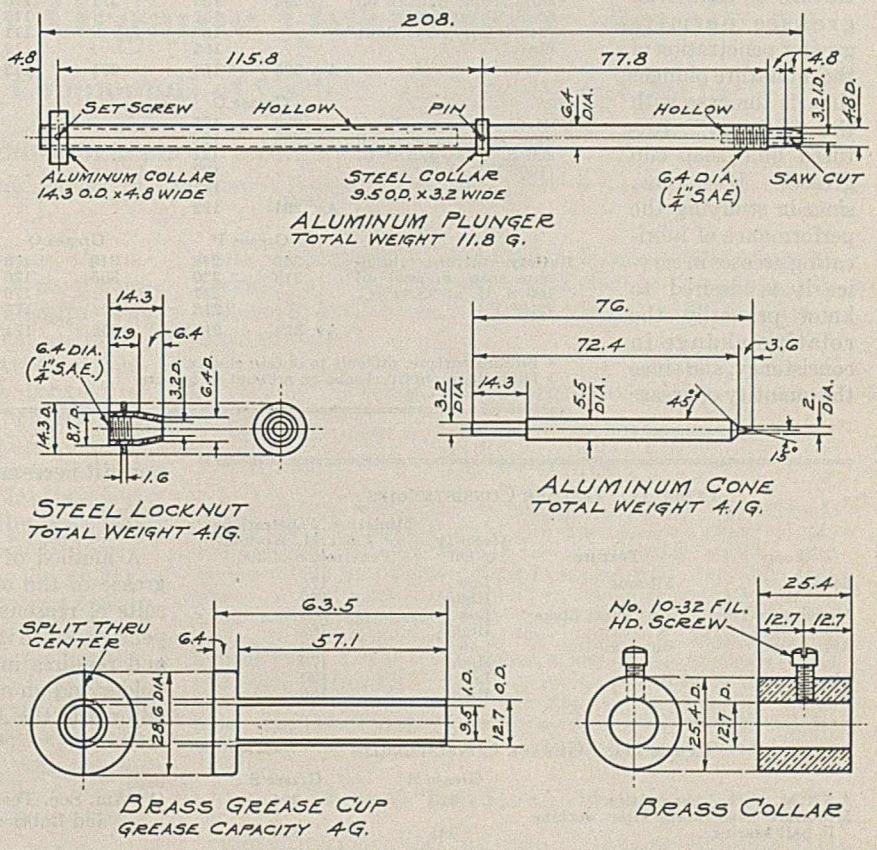

FIGURE 3. DETAILS OF CONSTRUCTION OF GREASE CUP, CONE, AND PLUNGER

All dimensions are in millimeters

the data submitted that the relationship, if any, varies with the texture of the grease, the particular soap used, the viscosity of the mineral oil, etc. In Table II miniature penetrations are shown for greases of different soaps, different textures, and different oils, but having approximately the same A. S. T. M. worked penetration of 300. For an A. S. T. M. worked penetration of about 300 the miniature penetration varies from 120 to 125 for a lime soap or aluminum soap grease of a buttery texture containing a low-viscosity oil to about 170 to 200 for a fibrous soda soap or mixed soda-lime soap grease containing an oil of either high or low viscosity. Apparently, therefore, the fibrous texture of soda soap greases permits greater penetration of the miniature plunger than is the case with the "buttery" textured lime soap cup greases. However, since in studying the performance of lubricating greases in service it is desired to know primarily the relative change in consistency, and since the quantity of grease available is too small for the A. S. T. M. penetrometer, the miniature penetrometer satisfactorily serves the purpose of determining the relative change in consistency of a grease after use compared to the original, unused product.

As an example of the application of the miniature penetrometer in practical evaluations, two greases having practically identical A. S. T. M. worked penetrations were used on the same ball bearing operating at 3,450 r.p.m. After such use less than 10 grams of grease were available for examination, insufficient for an A. S. T. M. worked penetration. The consistency of the used grease was therefore determined by the miniature penetrometer and it was found that a considerable difference in consistency of the two greases existed after use, in spite of the fact that the original A. S. T. M. worked penetrations were practically identical. Table III points this out and shows that the A. S. T. M. worked penetration does not necessarily predict the consistency of greases after use in ball bearings, nor is it necessarily a criterion of leakage tendency.

Table I. Comparative Consistency Determinations

(Penetrations of worked samples)

	Grease A		Grease B		Grease C	
	A.S.T.M.	Miniature	A.S.T.M.	Miniature	A.S.T.M.	Miniature
Buttery texture, calcium soap, mineral oil 300 S. U. at 37.78° C. (100° F.)	327	208	298	111	266	84
	323	209	292	108	262	86
	...	210	...	111	...	84
	...	209	...	110	...	85
	...	211	...	116	...	85
	...	211	...	118	...	..
	...	...	...	116	...	..
	Av. 325	210	295	113	264	85
	Grease D		Grease E			
Buttery texture, calcium soap, mineral oil 100 S. U. at 98.89° C. (210° F.)	299	167	278	104	...	..
	295	163	273	103	...	..
	...	166	...	103	...	..
	...	165	...	102	...	..
	Av. 297	165	276	103	...	..
	Grease F		Grease G			
Fibrous texture, sodium soap, mineral oil 300 S. U. at 37.78° C. (100° F.)	290	103[a]	232	57	...	..
	288	110[a]	225	57	...	..
	287	111[a]	...	57	...	..
	...	112[a]	...	62	...	..
	...	...	...	57	...	..
	...	...	...	58	...	..
	Av. 288	109[a]	229	58	...	..
	Grease H		Grease I		Grease J	
Fibrous texture, sodium soap, mineral oil 175 S. U. at 98.89° C. (210° F.)	348	291	286	131	214	60
	341	295	282	126	...	61
	...	298	...	130	...	63
	...	294	...	128	...	..
	Av. 345	295	284	129	214	61
	Grease K		Grease L			
Mixed sodium and calcium soaps, mineral oil 200 S. U. at 37.78° C. (100° F.), very short fibers	340	300[b]	312	177	...	..
	335	288[b]	316	167	...	..
	...	290[b]	315	167	...	..
	...	275[b]	...	177	...	..
	...	288[b]	...	177	...	..
	...	282[b]	...	...	...	..
	...	287[b]	...	...	...	..
	...	278[b]	...	...	...	..
	Av. 338	286[b]	314	173	...	..
	Grease M		Grease N			
Mixed sodium and calcium soaps, mineral oil 50 S. U. at 98.89° C. (210° F.), very short fibers	296	153	270	110	...	..
	293	155	271	110	...	..
	...	157	...	116	...	..
	...	160	...	111	...	..
	...	155	...	112	...	..
	Av. 295	156	271	112	...	..
	Grease O					
Buttery texture, aluminum soap, mineral oil 300 S. U. at 37.78° C. (100° F.)	289	125	...	...	...	..
	299	122	...	...	...	..
	...	121	...	...	...	..
	...	122	...	...	...	..
	...	121	...	...	...	..
	Av. 294	122	...	...	...	..
	Grease P		Grease Q			
Buttery texture, aluminum soap, mineral oil 100 S. U. at 98.89° C. (210° F.)	340	218	310	173	...	..
	318	220	305	176	...	..
	...	217	...	176	...	..
	...	218	...	175	...	..
	Av. 329	218	308	174	...	..

[a] Fibrous texture, difficult to obtain checks.
[b] Difficult to obtain checks on account of texture.

Table II. Grease Consistencies

Soap	Texture	Viscosity of Oil	Miniature Penetrations for A.S.T.M. Worked Penetration of 300
Soda	Fibrous	Low	175
		High	170
Mixed soda-lime	Very short fibers	Low	200
		High	160
Aluminum	Buttery	Low	125
		High	170
Lime	Buttery	Low	120
		High	165

Table III. Grease Consistencies

	Grease R	Grease S
A.S.T.M. worked penetration	323	324
Miniature penetration after working in ball bearing	345	456
Remarks	No leakage past bearing seal	Bad leakage past bearing seal

Summary

A method of obtaining penetrations of small samples of grease of the order of 4 grams is described, which gives results of reasonable reproducibility. The apparatus is inexpensive, since it utilizes the present A. S. T. M. penetrometer and requires in addition only a simply constructed grease holder and an aluminum plunger and cone. It is a valuable adjunct to the A. S. T. M. penetrometer.

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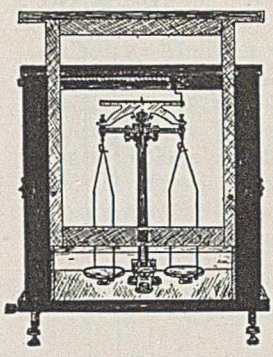
Received September 27, 1938. Presented before the Division of Petroleum Chemistry at the 96th Meeting of the American Chemical Society, Milwaukee, Wis., September 5 to 9, 1938.



Microchemistry

Microanalysis

SIMULTANEOUSLY with the recent emphasis upon microanalysis and microchemistry in this edition of INDUSTRIAL AND ENGINEERING CHEMISTRY, the question arose as to a proper definition which would guide authors, as well as ourselves, in deciding whether a given contribution belonged in that part of each issue devoted to microanalysis. We turned to officers of the Division of Microchemistry, and through a committee those prominent in the work were solicited for opinions. The result follows and represents the extent of the unanimous agreement on the part of the committee and the divisional officers. In allocating space for articles, we shall be guided by this definition. It will be noted that authors are expected to express an opinion in cases of possible doubt as to that portion of the ANALYTICAL EDITION in which their contribution should appear.

Microanalysis consists of techniques whose primary purpose is the ascertaining of chemical composition where the quantities dealt with are not more than one tenth as large as in customary laboratory practice. Manipulative and observational techniques, which even though nonchemical are peculiar to or especially important to microanalysis, shall be included.

The editor of the journal shall interpret this definition and, guided by the expressed opinions of the author and the reviewer, shall allocate papers to the microchemical or the general section of the journal.

Microtechnique of Organic Qualitative Analysis

Classification Reactions of Compounds of Carbon, Hydrogen, and Oxygen

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IN ALL schemes of qualitative organic analysis, the unknown substance is classified as a definite type of compound by the use of classifying reactions. These reactions are usually carried out in a definite order, so that each reaction will identify but one or, at most, two types of compounds. In schemes which use the solubility behavior of organic compounds, the number of classification reactions which must be carried out is considerably reduced. In all other respects, however, the schemes are the same as those which are based on the elementary composition of the substance, such as the scheme of Mulliken and Huntress (*17*).

The next step, therefore, in the development of the microtechnique of qualitative organic analysis, after the preliminary examination and solubility tests described in previous papers (*8*, *21*) is to work out the procedure for the so-called classification reactions for compounds containing carbon, hydrogen, and oxygen. These reactions are the fuchsin test for aldehydes, Molisch test for carbohydrates, titration for acids, ferric chloride and alkali solubility for phenols, saponification for esters, phenylhydrazine test for ketones, and sodium test and solubility test for alcohols. The tests are carried out in that order until a positive test is obtained which places the substance in that group.

The following classification reactions are for compounds containing carbon, hydrogen, and oxygen only. The only reaction which may be called a real classification test for compounds containing other elements is the titration of nitrogen-containing compounds. This test will be described in a subsequent paper.

Fuchsin Test for Aldehydes

Emich (*4*) mentions this test briefly but leaves the method of applying the test to the reader.

The authors carried out the test in the case of water-soluble substances by placing 2 drops (from a capillary pipet) of the reagent (prepared according to the directions of Mulliken and Huntress, *17*) in a shallow depression of a white porcelain spot plate. A tiny drop (0.02 to 0.05 cu. mm.) of the substance is added to the reagent drop, stirring if necessary. A distinct pink, red, or purple color develops within 2 minutes if the substance is an aldehyde. In the case of solids, a tiny crystal is placed in the spot-plate depression. To this is added just enough aldehyde-free alcohol to dissolve it and then the reagent as above. With water-insoluble liquids the authors proceeded as with solids, using 0.02 to 0.05 cu. mm. of the substance.

Molisch Test for Carbohydrates

Emich mentions this test also (*5*) but as Huntress (*17*) points out, the alcoholic reagent Emich employs as well as his procedure for mixing the reagents may lead to incorrect

conclusions. The authors have therefore taken the method of preparing the reagent which Huntress recommends—that is, a freshly prepared chloroform solution of α-naphthol. The test is carried out as follows:

End *A* (Figure 1, *a*) of the capillary (1-mm. bore, 100-mm. length) is dipped into the 1 per cent sugar solution until a droplet about 0.5 cm. long has risen in the capillary. If too much is taken, the excess can be removed by inserting the point of a triangular piece of filter paper into this end of the capillary (Figure 1, *b*). The capillary is then inclined towards *B* and the droplet allowed to flow a short distance towards the middle of the tube. The finger is then placed over end *B* and end *A* dipped into the reagent solution (a drop on the slide will do, Figure 1, *c*). When the pressure of the finger is gradually released, the reagent (1 to 2 mm.) rises in the tube. Both droplets are then allowed to slide to the middle of the capillary. End *A* is then closed with the finger and end *B* dipped into concentrated sulfuric acid to a depth of 1 cm. The finger is then removed from end *A*, the acid enters the tube for a distance of 1 cm., and the end is closed again with the finger. All the droplets are then allowed to slide to the middle of the capillary (Figure 1, *d*). End *B* is wiped off and sealed in the flame. All droplets are then centrifuged briefly (1 or 2 turns) to end *B*. This brings the sulfuric acid on the bottom, followed by the reagent, and then the test solution. Thus mixing of the reagent and sugar solution is accomplished but the proper contact between acid layer and reagent-substance layer is secured. In the presence of carbohydrates, a red ring forms at the acid-reagent interface. Then the solutions are mixed by means of a glass thread, whereupon a violet color appears throughout the liquid. On dilution a violet precipitate may appear. The sealed end is then cut off and the liquid blown out into a depression of a spot plate. Excess concentrated ammonia is added. A yellow-red color results.

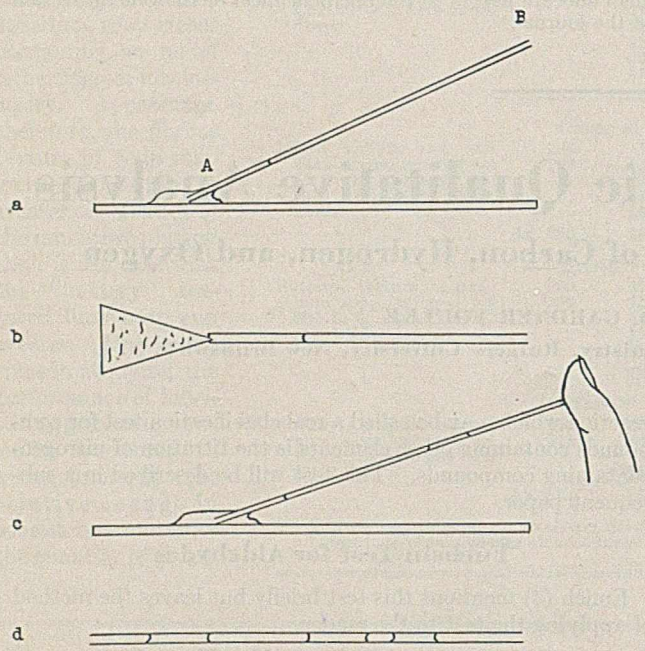

FIGURE 1. CARBOHYDRATE TEST

Ten carbohydrates were tested in this way and correct results obtained in each case.

Titration of Acids

The titration of a few milligrams of acids has been described by a number of authors (*3*, *9*, *18*). Since, however, according to Mulliken and Huntress, it is necessary to proceed with the titration under certain very definite conditions in order to classify the substance properly as an acid and to avoid the possibility of including phenols, esters, anhydrides, or lactones in this group, it was necessary to reproduce these conditions as nearly as possible on a micro scale. At the same time the comparatively elaborate equipment and procedure used in the ordinary quantitative microtitrations should be avoided, in order to adhere to one of the principles of the authors' work—that is, to keep the apparatus as simple as possible.

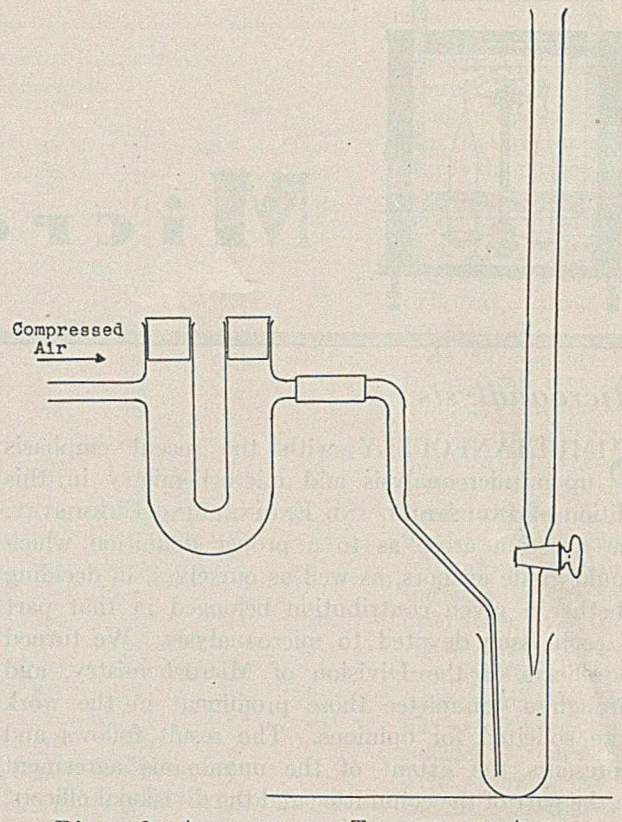

FIGURE 2. APPARATUS FOR TITRATION OF ACIDS

The sample, if a solid, is weighed out either on a Salvioni balance or a good analytical balance. About 5 mg. are taken. In the case of liquids the density of which is known, a definite volume can be taken in a capillary. The sample is dissolved in 2 ml. of water in a 7- to 10-ml. microbeaker made from the bottom of a test tube. One drop of phenolphthalein indicator solution is added. A fine capillary connected to a source of compressed air through a soda-lime tube is dipped in the solution, (Figure 2). It is placed to one side so that the rising stream of bubbles causes thorough circulation. The carbon dioxide–free air forms a cover over the liquid surface and prevents the absorption of carbon dioxide from the atmosphere. The tip of a 10-ml. buret (in 0.05 ml.) is dipped into the beaker (but not into the solution) opposite the air inlet capillary. The 0.02 *N* sodium hydroxide is added dropwise at such a rate that the color due to one drop is dissipated before the next drop is added. The end point is reached when the color persists for more than 1 minute. In the case of water-insoluble substances, 2 ml. of alcohol are used in place of the water as solvent.

According to Huntress (*17*), a substance is considered an acid if it gives a sharp end point (one drop of alkali causing a permanent color change) and a normal color change (full pink). A series of titrations on water-soluble and insoluble substances showed that the conditions described are satisfied by the microprocedure.

Ferric Chloride Test for Phenols

Emich (*6*) mentions this test but gives no details for carrying it out. He lists the colors obtained with some phenols (see also Meyer, *15*). Kisser and Kondo (*14*) describe a spot-test procedure which, however, is limited in its application. The authors used a spot plate and the reagent de-

scribed by Mulliken and Huntress. The droplet of reagent used should be small. The characteristic color changes can readily be observed.

According to Mulliken and Huntress, if no color is obtained in the above test in the case of solids, a test of the solubility first in water and then in 5 per cent sodium hydroxide must be applied. This test can be carried out by the solubility testing procedure described in a previous paper (*21*).

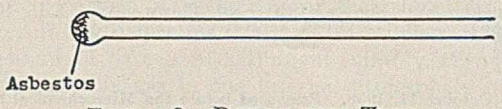

FIGURE 3. DISTILLATION TUBE

If the substance is found to be soluble in water in the cold and to give no color with ferric chloride, it is not a phenol. If it is soluble in five per cent sodium hydroxide it should be regarded as a member of this group.

Saponification of Esters

The saponification procedures described in the microchemical literature are almost exclusively restricted to heavy fats and oils (*12*, *16*, *20*). While there was no doubt that the usual saponification methods using alcoholic alkali could be readily adapted to microwork, the authors were so impressed by the advantages of the method of Redeman and Lucas (*19*), using a diethylene glycol solution of the alkali, that they determined to develop a microprocedure based upon it. Again they found that despite the apparent simplicity of the macroprocedure, the micromethod was still simpler.

A distillation tube is prepared by blowing a bulb about 6 to 7 mm. in diameter at the end of a Pyrex tube of 4- to 5-mm. bore and about 100 mm. long (Figure 3). This bulb is half filled with ignited asbestos and 30 cu. mm. of the diethylene glycol solution made up as described by Redeman and Lucas are introduced directly into the bulb by means of a capillary pipet. Then 10 cu. mm. of the ester are introduced in the same way. Care should be taken that no liquid touches any part of the walls of the tube except the bulb. The capillary pipet should be wiped off on the outside before it is introduced into the distillation tube. If the liquids do not soak into the asbestos, brief centrifuging will bring this about.

The tube is then placed in a Benedetti-Pichler heating block (*2*), and the block and tube are heated slowly until a condensate appears at the portion of the tube projecting from the block. The rate of heating can then be increased until a definite ring of condensate forms. Redeman and Lucas have determined the reaction times of various esters and found that all reactions, when carried out on a large scale, are complete within 2 minutes; hence, by the time the first condensate appears the reaction is complete. After formation of the ring of condensate, heating is continued until the ring is about 1 or 2 cm. from the block. It is then taken up in a capillary pipet. Sometimes no definite ring forms, only a larger number of drops. These can be gathered together and picked up by the capillary pipet just as readily.

Since there is always water in the reagents even if it is not added in making up the alkali, some water will distill with the alcohol. In the case of water-insoluble alcohols this is not serious, since the combined water and alcohol condensate in the capillary pipet can be separated into its components by sealing the fine end of the pipet and centrifuging. The capillary is cut at the interface and the alcohol transferred to a boiling point tube. In the case of water-soluble alcohols, some help is obtained from the fact that these boil at temperatures below the boiling point of water. Thus the first condensate is the alcohol, which, with care, can be collected without the water. In any event the alcohol is dried as described by Benedetti-Pichler (*11*). The boiling point can then be determined as described by Emich (*7*) and the alcohol thus identified. The acid part of the ester is identified by adding a drop of water and a drop of alcohol to the residue from the distillation. After stirring and centrifuging, the liquid is transferred to a centrifuge cone by means of a capillary pipet. A drop of phenolphthalein is added and the solution acidified with sulfuric acid. Centrifuging removes the potassium sulfate and the clear liquid can be siphoned off and analyzed for the acid, as will be described in a subsequent paper.

Acid Anhydrides and Lactones

There is no classification reaction for these compounds, but if the saponification equivalent is less than 500 and no alcohol is obtained in the foregoing test the compound is placed in this group.

Phenylhydrazine Test for Ketones

Procedures for carrying out this test have been described by Behrens (*1*), Emich (*4*), and Garner (*10*). Garner uses up to 50 mg. in a "microflask" or "anilide tube"; the others employ microscope slides. Griebel and Weiss (*13*) describe a technique which is limited to easily volatile substances. The authors carried out the test in capillary tubes. They claim no advantages for their method except that it makes it possible to recover the product more readily for further identification. About 0.5 cu. mm. of reagent is drawn into a capillary tube (1-mm. bore) and allowed to slide to the middle. About 5 times as much of the sample is drawn in at the other end of the capillary. The first end is sealed and the reagent and sample are centrifuged to this end. An immediate precipitate is obtained with ketones.

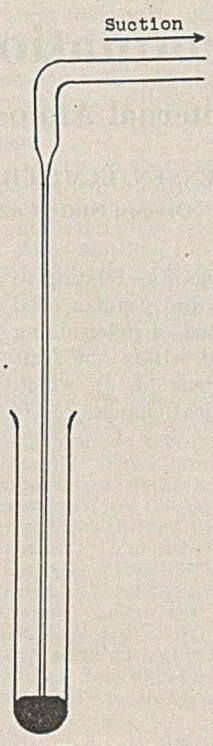

FIGURE 4

Alcohol Test with Sodium

Mulliken and Huntress place a compound in this group if it has failed to give a positive reaction in the preceding tests and if it is soluble in 50 parts or less of water at 20° C. (see also *21*).

If the substance does not dissolve in 50 parts of water at 20° C. the sodium test is applied.

The reagent is prepared by melting some sodium in a hard-glass test tube. A glass tube is drawn out into a thin-walled capillary of about 0.5-mm. bore and about 150 mm. long. Without cutting the capillary from the wide tube, the former is dipped into the molten sodium and suction cautiously applied (Figure 4). The sodium will rise in the tube and is then allowed to cool and congeal. Short pieces of the filled capillary are used and are cut off just before use to ensure a clean surface of the sodium.

The test is carried out by placing a drop of the alcohol on a slide. A piece of the sodium capillary is laid on the slide so that the fresh-cut end is in the center of the drop. The whole setup is placed under a lens or low-power microscope. If the substance is an alcohol, bubbles of hydrogen will appear issuing from the capillary.

Hydrocarbons and Ethers

If the substance does not give a positive result in any of the preceding tests, it is placed in the group of ethers and hydrocarbons.

Acknowledgment

The authors wish to express their thanks and appreciation to E. H. Huntress of the Massachusetts Institute of Technology for his kindness in placing at their disposal material which he has not published as yet and for a copy of his unpublished revision of Mulliken's book on qualitative organic analysis.

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Determination of Ethylene

In the Internal Atmosphere of Plant Tissues

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DURING certain physiological investigations relating to the handling and storage of apples and pears, a need developed for an accurate chemical method of determining the small amounts of ethylene contained within the fruit tissues. Since these fruits produce ethylene (*5*, *7*), which is definitely known to affect certain chemical changes (*6*, *8*) that are associated with the ripening and storage of fruit, a means of obtaining data of this nature is desirable.

Although ethylene has been identified as a constituent of fruit emanations (*5*, *11*) and has been semiquantitatively estimated, the procedures used would not lend themselves to the development of a rapid and accurate method for the de-

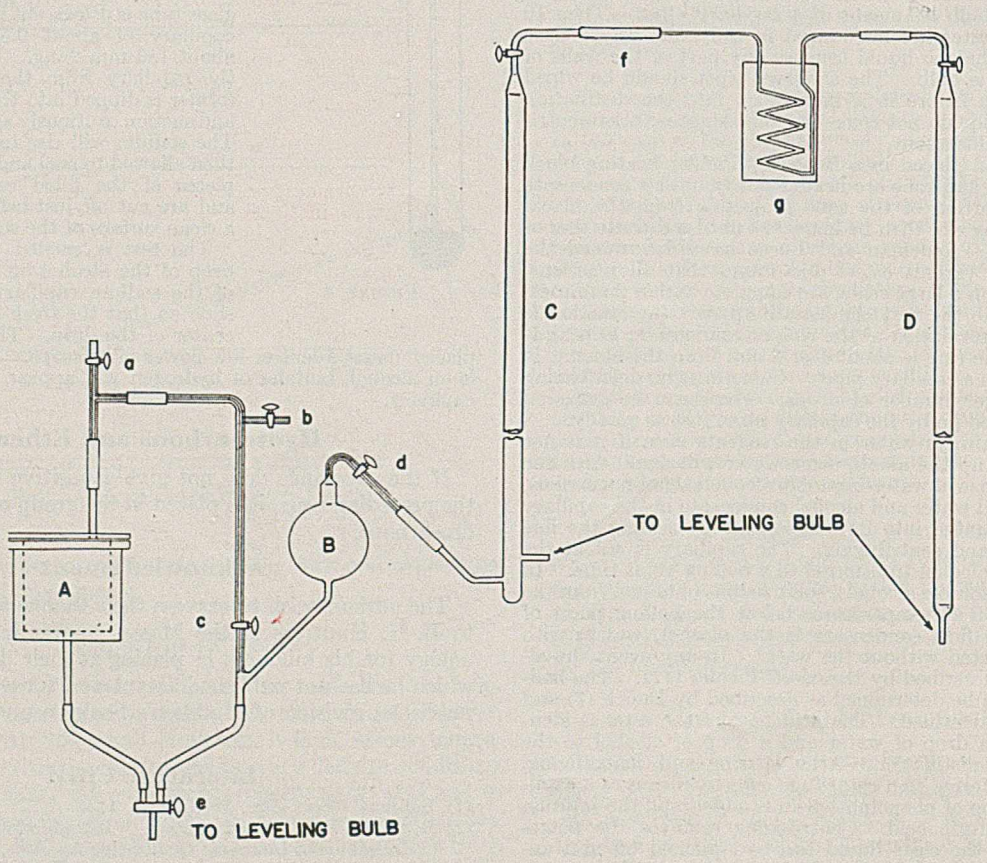

FIGURE 1. DIAGRAM OF APPARATUS

termination of the gas contained in the internal atmosphere of the tissue.

The amount of ethylene that occurs in plant tissues is thought to be very small (*4*). Nelson (*10*) reports the ethylene content of McIntosh apples as 0.12 mg. per kilogram. Since 1 kg. of fruit tissue will contain on the average from 300 to 600 ml. of total gas, this represents a dilution in the order of one part in four thousand. If these values are approximately correct, it would appear that a method which could accurately determine 0.001 ml. of ethylene in a dilution of 40 ml. (1 part in 40,000) might suitably serve for the estimation of this gas in the internal atmosphere of fruit tissue.

A survey of the possible chemical reactions which might serve as a basis for a microdetermination indicated that bromination or oxidation with potassium permanganate would be the most promising. A method using permanganate has recently been described by Nelson (*10*).

Extensive preliminary experiments conducted in this laboratory indicate that the reaction of ethylene with permanganate, besides being affected by traces of acids, bases, or organic impurities, does not always proceed to a definite quantitative product—viz., glycol—and as a result only the roughest of approximations may be attained by its use.

Bromination on the other hand has the distinct advantage that the reaction does not proceed readily beyond the formation of ethylene dibromide and is not so easily influenced by small amounts of foreign materials. For these reasons a simple method modified from the macrodetermination of Davis, Crandall, and Higbee (*3*) was adopted.

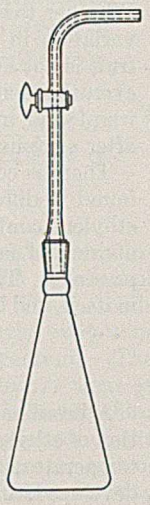

FIGURE 2

Apparatus

The apparatus consists of three units: an extractor, a purification train, and a reaction flask.

EXTRACTOR. The extractor (Figure 1) was constructed on the same principle as a Töpler pump and consisted of two parts: a chamber, *A*, and a pump, *B*. The extraction chamber, *A*, was constructed from an iron pipe 8.9 cm. (3.5 inches) in inside diameter and 7.6 cm. (3 inches) over-all. To the bottom was welded a steel plate fitted with a 0.64-cm. (0.25-inch) steel tube which served as an inlet for the confining fluid. A 1.25-cm. (0.5-inch) iron collar was welded to the opposite end and then carefully machined to give a smooth surface. The cover, equipped with an exit tube, was made from a 0.64-cm. (0.25-inch) steel plate carefully polished and fitted to the collar. A tight connection capable of maintaining vacuum was obtained by the use of a greased rubber gasket. The cover was held in position by means of a heavy screw clamp like those used on specimen jars.

The pumping compartment, *B*, was constructed from a 250-ml. Pyrex bulb and was connected to the extraction chamber with 1-mm. capillary tubing. Stopcock *c* was used to control the flow of gas between these two units. Stopcock *a* provided a means of releasing the vacuum, while *b* led to a manometer for measuring the pressure in the extraction chamber. In order to ensure flexibility a rubber connection was placed between stopcocks *a* and *b*. By means of stopcock *e*, the same leveling bulb was used for forcing mercury into either *A* or *B*.

A nitrometer, *C*, which served to trap the extracted gases, was connected to *B* through stopcock *d*.

PURIFICATION. The purification unit consisting of a small Desicchlora tube, *f*, and a copper coil, *g* (2 mm. in inside diameter × 100 cm.), immersed in a solid carbon dioxide–ether mixture, was connected between nitrometer *C* and gas buret *D*. The total volume of the tube and coil was 5.3 ml. Buret *D*, equipped with a leveling bulb containing mercury, served to store and measure the purified gas prior to analysis.

TABLE I. RECOVERY OF ETHYLENE ADDED TO APPLE TISSUE

Ethylene Taken	Ethylene Recovered
Ml.	*Ml.*
0.058	0.051
0.057	0.059
0.058	0.056
0.058	0.062
0.061	0.061

REACTION FLASK. The reaction flask (Figure 2) was constructed from a 50-ml. Erlenmeyer to which was sealed a $^{12}/_{30}$ standard taper and a capillary stopcock.

Operation

A weighed amount of tissue (either whole or cut) was placed in chamber *A*. The cover was then clamped in position and connected to the pumping compartment. With stopcock *a* open *A* was filled with mercury from the leveling bulb; *a* was then closed and the mercury allowed to drain away, leaving the tissue in a Torricellian vacuum. By means of stopcock *e* and the same leveling bulb, chamber *B* in turn was filled with mercury and evacuated. Nitrometer *C* was then filled with mercury, over which was placed 1 ml. of 2.5 per cent ammonium hydroxide solution.

The gas in the storage flask was now completely removed and transferred to nitrometer *C* by merely raising and lowering the leveling bulb and operating stopcocks *c* and *d*. This process was continued until no further gas could be extracted.

After standing for 15 minutes in nitrometer *C*, the gas was passed slowly (approximately 4 ml. per minute) through the purification train to the measuring buret, *D*, where it remained until removed for analysis.

The reaction flask (Figure 2) was then charged with 5.00 ml. of 0.0025 *N* potassium bromate (measured with a microburet) and 0.5 ml. of 6 *N* sulfuric acid, and then partially evacuated. Buret *D* was detached and approximately 40 ml. of sample were transferred to the reaction flask. One milliliter of 0.1 *N* potassium bromide was finally introduced without releasing all the vacuum. This mixture was shaken vigorously for 15 minutes on a mechanical shaker and then 1 ml. of 0.1 *N* potassium iodide was introduced by means of the residual vacuum. The iodine liberated was titrated with 0.0025 *N* sodium thiosulfate from a microburet. The amount of potassium bromate used for bromination was determined by the difference between the blank run (using air) and the actual determination. Duplicate blank runs checked consistently within 0.02 ml. One-tenth milliliter of 0.0025 *N* potassium bromate is equivalent to 0.0028 ml. of ethylene at normal temperature and pressure.

In all determinations a correction was made for the gases remaining in the purification train.

Discussion and Results

EFFICIENCY OF EXTRACTION. To determine the efficiency of the apparatus for extracting ethylene from fruit tissue, blank determinations were made using ethylene-air mixtures. Approximately 100 grams of sliced apple were placed in the storage compartment and all the free ethylene present was removed by extraction. Small known quantities of ethylene were introduced into the chamber and sufficient time was allowed to ensure diffusion throughout the fruit and container. Forty milliliters of air were then introduced (that amount being the approximate volume of gas taken for analysis). This gaseous mixture was then removed and analyzed in the manner previously described.

From the results of a number of determinatious, shown in Table I, it is apparent that small quantities of ethylene can be recovered quantitatively when added to apple tissue. Furthermore, consecutive tests on fresh samples have failed to show any trace of ethylene after the first extraction. Ethylene did not appear to be given off in a definite pressure range.

The advantages of this type of extraction are: It attains a practically complete removal of gas; it permits the study of either whole or cut tissue; and it is equipped to measure the

TABLE II. MICRODETERMINATION OF ETHYLENE

Ethylene Taken	Ethylene Found
Ml.	*Ml.*
0.0026	0.0025
0.006	0.006
0.011	0.011
0.029	0.028
0.052	0.049
0.066	0.066
0.063	0.061

TABLE III. ETHYLENE CONTENT OF INTERNAL ATMOSPHERE OF FRUIT AND VEGETABLE TISSUES

Kind of Fruit	Method of Sampling	Ethylene Found
		Ml./100 g.
Apple:		
Gravenstein	Longitudinal sectors	0.019
		0.016[a]
	Whole apple	0.026
		0.040
		0.063
		0.039
		0.022
	Average of 6 whole fruits	0.038
Red June	Longitudinal sectors	0.008
	Average of 6 whole fruits	0.012
Bartlett Pear	Whole fruit	0.015
		0.029
		0.010
		0.008
		0.020
		0.012
Peaches:		
Hale	Whole fruit	0.037
Crawford		0.012
Tomatoes	Whole fruit	0.010
Cantaloupe	Gas from cavity	0.006
	Longitudinal sectors	0.002
Potatoes	Long sectors	<0.001
Bananas	Long sectors	<0.001

[a] Sectors taken from same apples 3 hours later.

pressures at which the gases are extracted. Although several methods (*1*, *2*, *9*, *10*) for the removal of internal gases from fruit tissue are described in the literature, none attained all these objectives.

EFFECTIVENESS OF PURIFICATION TRAIN. In order to determine the ethylene content in the vapors derived from fruit tissues it was necessary to remove the other components, such as aldehydes, esters, and alcohols.

Preliminary experiments carried out under the conditions specified for analysis showed (1) no bromination of ethyl acetate, (2) slight bromination of ethyl alcohol, and (3) considerable bromination of acetaldehyde.

To eliminate these substances, 1 ml. of 2.5 per cent ammonium hydroxide was placed in nitrometer *C* over which the vapors were permitted to stand for 15 minutes. Blank determinations showed that this treatment completely removed both acetaldehyde and alcohol vapors, even when present in great excess over that found in fruit vapors. In using this procedure, however, care must be taken to prevent any of the ammonia from entering the analytical flask, since it would alter the acidity of the reaction mixture. To ensure the removal of ammonia and possibly other active agents, the extracted gas was finally passed through a Desicchlora tube and a cold trap.

Since it appears that ethylene is the only unsaturated gas present in apple (*5*), pear (*7*), and banana (*11*) vapors, no special precautions were taken to remove possible traces of acetylene or propylene.

ACCURACY OF THE ANALYTICAL METHOD. To determine the accuracy of the analytical procedure, a number of typical runs were made with pure ethylene and are tabulated in Table II.

ETHYLENE CONTENT OF THE INTERNAL ATMOSPHERE OF PLANT TISSUE. Using the procedure outlined, the ethylene content of various kinds of ripe fruit material was determined. In some of these analyses, gas samples were taken from whole fruits; in others, from slices of eight or more selected specimens. In the case of the cantaloupe, the gas sample from the cavity was taken by inserting a glass tube and extracting the gas directly into the buret. The results are tabulated in Table III.

Table III shows that there is considerable variation in the ethylene content of individual fruits taken from the same lot. Ripe Gravenstein apples showed a variation of 0.022 to 0.063 ml. per 100 grams of tissue. Bartlett pears showed a variation of from 0.008 to 0.029 ml. Some of the data indicate that gas samples taken from whole fruits gave higher ethylene values than similar samples taken from cut fruit. Whether this difference is due to loss of gas in cutting or to variation in the ethylene content in different parts of the fruit is not known at the present time. It is apparent, however, that some gas is lost from cut tissue, since more ethylene was found in cut Gravenstein tissue analyzed immediately after sampling than in similar tissue analyzed 3 hours later.

There is considerable variation in the amount of ethylene found in different kinds of fruits and vegetables. Since the ethylene content of a given variety of fruit is dependent on a number of factors, these values are merely indicative of its presence. The variation in each type of material is a study in itself, and beyond the scope of this paper.

Summary

A bromination micromethod for the accurate determination of ethylene within a range of 0.001 to 0.06 ml. at normal temperature and pressure in a volume of 35 to 40 ml. has been developed, and a new apparatus devised for the complete removal of internal gases from plant tissue.

A number of analyses have been carried out to show the presence of unsaturates (ethylene) in various kinds of tissue in quantities measurable by this method.

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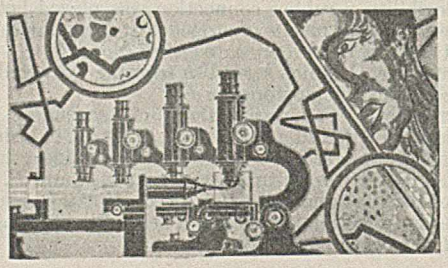

Qualitative Separations on a Micro Scale

Separations in the Alkaline Earth Group

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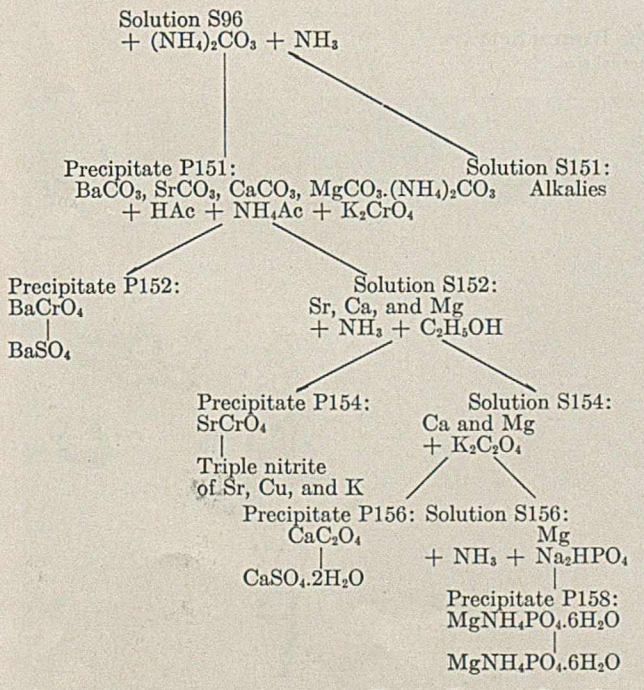
The procedure of Noyes and Bray for the separation and analysis of the alkaline earth group has been applied on a micro scale. The quantities of the reagents have been chosen so as to permit the analysis of 1.2 mg. of nonmetallic materials or 0.6 mg. of metals and alloys. The perchlorate ion, introduced by the use of perchloric acid as solvent, is removed before precipitation of the alkaline earth group.

THE present paper deals with the elements commonly included in the alkaline earth group which consists of barium, strontium, calcium, and magnesium. The methods follow the scheme of Noyes and Bray (*5*) with the exceptions of the confirmatory tests for barium and strontium. The working technique and apparatus are essentially the same as those suggested for the separations of members of the third analytical group (*3*). To facilitate the precipitation of the whole group as carbonates, of strontium chromate, and of magnesium ammonium phosphate the use of the vibrating armature of an ordinary door bell was found to be effective.

The tabular outline shows the general scheme for the separations. The numbers of the precipitates and solutions correspond to the numbers of the paragraphs in Noyes and Bray (*5*) in which the analogous macroprocedures are described.

Procedure



PRECIPITATION OF THE ALKALINE EARTH GROUP. In order to remove perchlorate ion and the large quantity of ammonium salts, evaporate ammonium sulfide filtrate S96 to dryness in small portions in a cone over a steam bath. Evaporate twice with 25-cu. mm. portions of concentrated hydrochloric acid. Extract the residue with three 50-cu. mm. portions of water, separating any insoluble residue by centrifuging after each addition and evaporate the combined extracts over steam in small portions in a platinum crucible. Moisten the residue with 10 cu. mm. of 6 *M* ammonium chloride solution and evaporate to dryness. Heat the crucible at 140° C. for 10 minutes. Then place the crucible on a silica triangle and heat cautiously with a small flame until fumes are no longer given off. Finally heat the crucible just to the point of dull redness and discontinue further heating. Extract the residue with several 10-cu. mm. portions of water, add the solution to a cone, evaporate to dryness, and dissolve in 10 cu. mm. of water. The solution should be clear at this point. If it is not, separate solution and residue by centrifuging.

To the solution add 15 cu. mm. of ammonium carbonate reagent (*5*) and 15 cu. mm. of 95 per cent ethyl alcohol. If the precipitate is large, add 15 cu. mm. more of each of these solutions. Stir with a glass thread, attach to the vibrator, shake intermittently for 10 minutes, and centrifuge. Remove the solution and wash the precipitate with a little ammonium carbonate reagent.

PRECIPITATION OF BARIUM. Add to precipitate P151 in the cone 5 to 15 cu. mm. of 6 *M* acetic acid and warm on the steam bath until the carbonates are dissolved. Evaporate the acetic acid solution to dryness over the steam bath in a current of air. To the residue add 10 cu. mm. of water, 10 cu. mm. of 3 *M* ammonium acetate, and 2 cu. mm. of 6 *M* acetic acid. Heat the mixture on the steam bath and add to it, in portions of about 0.3 cu. mm., 3 cu. mm. of 1.5 *M* potassium chromate, shaking after each addition. If the precipitate is large, add 2 cu. mm. more of the potassium chromate solution. Allow to stand on the steam bath for 5 minutes. A yellow precipitate shows the presence of barium. Centrifuge and separate solution and precipitate. Wash the precipitate with a little water and set it aside for later use.

Estimate the amount of barium present by comparing the size of the precipitate with a known amount of barium chromate precipitate obtained by treating a solution of barium nitrate in the same manner as the unknown solution.

PRECIPITATION OF STRONTIUM. To solution S152 which may contain strontium, calcium, and magnesium add 6 *M* ammonia until the solution turns yellow and then 5 cu. mm. more. Heat in a water bath to 60° to 70° C. and add in three portions 15 cu. mm. of ethyl alcohol, shaking or stirring after each addition if a precipitate occurs. If a large precipitate results, add 3 cu. mm. more of potassium chromate solution and 15 cu. mm. of ethyl alcohol. Cool the solution, oscillate on the vibrator one minute, allow to stand one minute, and centrifuge. Estimate the amount of precipitate as in the case of barium chromate. Separate solution and precipitate, but do not wash the latter.

The necessity of the performance of a confirmatory test for barium depends upon the quantity of strontium found (*5*). If strontium is either absent or present in a small quantity, formation of precipitate P152 is sufficient proof of the presence of barium.

CONFIRMATION OF BARIUM. Dissolve barium chromate precipitate P152 in a cone with 5 to 10 cu. mm. of hydrochloric acid. Add 5 cu. mm. of 4 *M* sulfuric acid, stir, and centrifuge. Remove the solution and wash with three portions of 0.1 *M* nitric acid to remove all traces of calcium, as these interfere with the later formation of barium sulfate crystals. To the precipitate add such an amount of concentrated sulfuric acid as to form a mixture containing 5 micrograms of barium per cubic millimeter of solution. Stir the mixture, remove 5 cu. mm. of the slurry obtained with a capillary pipet, and place on a slide. Heat the slide over a microburner until dense white fumes are evolved and allow to cool. If crystals do not appear in a few minutes, breathe once or twice over the solution. If crystals are too small, repeat the heating of the test drop as described above. The characteristic forms of the crystals of barium sulfate and strontium sulfate are shown in the photomicrographs (Figures 1 to 4). Sulfuric acid which has stood in a small reagent bottle for some time should not be used for this test (*4*).

CONFIRMATION OF STRONTIUM. To strontium chromate precipitate P154 add 25 cu. mm. of 3 *M* sodium carbonate solution and heat on the steam bath with stirring for 10 minutes. If more than 50 micrograms of strontium are present, repeat the treat-

ment with another 25-cu. mm. portion of the sodium carbonate solution. Centrifuge, separate the solution and precipitate, and wash with three 5- to 10-cu. mm. portions of 3 *M* sodium carbonate solution. Add sufficient hydrochloric acid to dissolve the precipitate and evaporate in small portions on a slide. Test the residue for strontium by the method of Adams (*1*). [The nitrite reagent is prepared by mixing equal volumes of potassium nitrite solution and acetate buffer solution. The former contains 500 grams of potassium nitrite in 1 liter. The latter is prepared by adding 450 grams of sodium acetate trihydrate (or 325 grams of potassium acetate) and 100 ml. of glacial acetic acid to sufficient water to make 1 liter of solution. The mixed reagent decomposes slowly, but if kept in a stoppered microcone it may be used for several days.]

Moisten the residue with a solution of cupric nitrate or cupric acetate which contains a quantity of cupric ion equal to five times that of the strontium present. Evaporate the mixture to dryness. When the slide has cooled to room temperature treat the residue with a small volume of the new nitrite reagent (*1*). The appearance of small green squares—probably a triple nitrite of strontium, copper, and potassium—confirms the presence of strontium. The use of transmitted light of high intensity is essential for the recognition of the color of the crystals under the microscope. The green squares of the strontium compound separate a few minutes after addition of the nitrite reagent. Barium, calcium, and magnesium do not give the test. If a nitrite solution containing 1,000 grams of potassium nitrite per liter is used, the strontium test is more sensitive, but calcium interferes by forming a few green crystals. Barium does not form green crystals but interferes with the strontium test if the quantity of barium present is ten times that of strontium.

Separation of Calcium from Magnesium. Add 50 cu. mm. of water to solution S154, stir, add 3 cu. mm. of 1.5 *M* potassium oxalate, and unless a precipitate has already occurred, let the mixture stand about 15 minutes. If a precipitate separates, heat the mixture to 70° to 80° C. and add gradually 3 to 10 cu. mm. more of potassium oxalate solution, adjusting the total volume of the reagent to the size of the carbonate precipitate. Heat for 5 minutes, centrifuge at once, and separate the solution with a capillary siphon. Wash the precipitate with two portions of water. Estimate the amount of calcium present by comparing with a known amount of calcium oxalate.

Confirmation of Calcium. Dissolve precipitate P156 with such an amount of 6 *M* hydrochloric acid as to form a solution

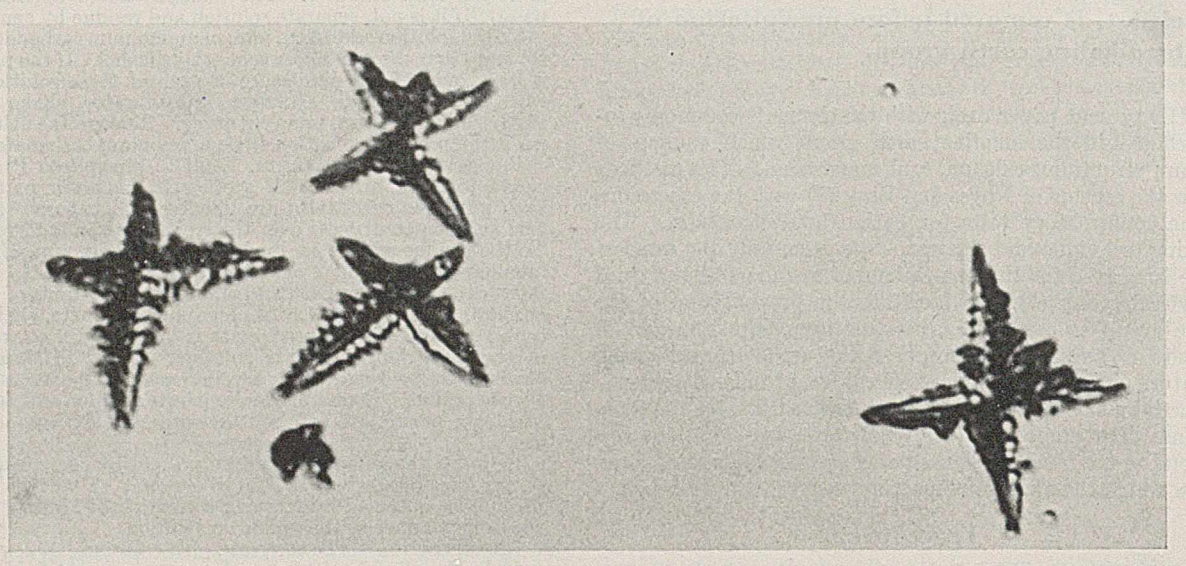


Figure 1. Crystals of Barium Sulfate
1,180 times actual size

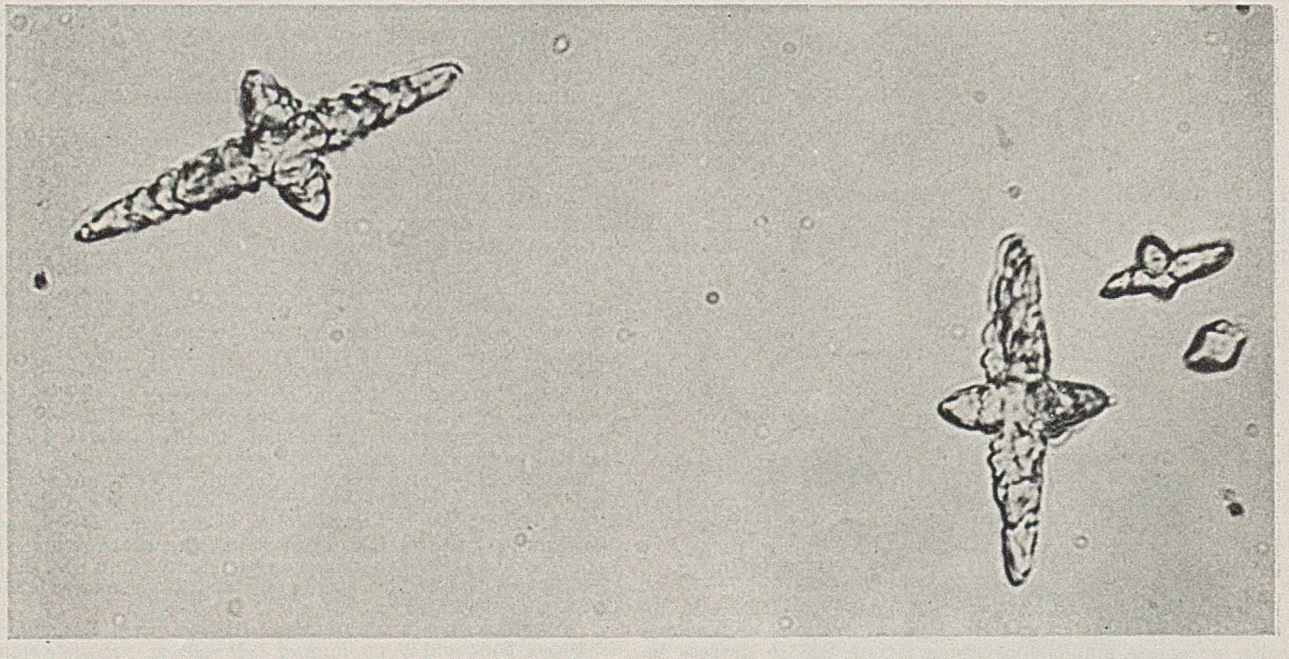


Figure 2. Strontium Sulfate Crystals
800 times actual size

containing approximately 5 micrograms of calcium per cubic millimeter of solution. Place the solution on a slide and near it place 1 cu. mm. of 4 *M* sulfuric acid. By means of a glass thread draw a narrow channel of liquid connecting the two solutions. If calcium is present, a microscopic examination of the test solution will show the gradual appearance of crystals of calcium sulfate dihydrate such as those shown in the photomicrograph.

In very dilute solutions the crystals form only on complete evaporation of the test drop.

DETECTION OF MAGNESIUM, To solution S156 add 5 cu. mm. of 15 *M* ammonia and 25 cu. mm. of 0.3 *M* disodium phosphate. Cool, and allow to stand 0.5 hour with frequent shaking and oscillating on the vibrator. The presence of magnesium is indicated by the appearance of a white precipitate of magnesium am-

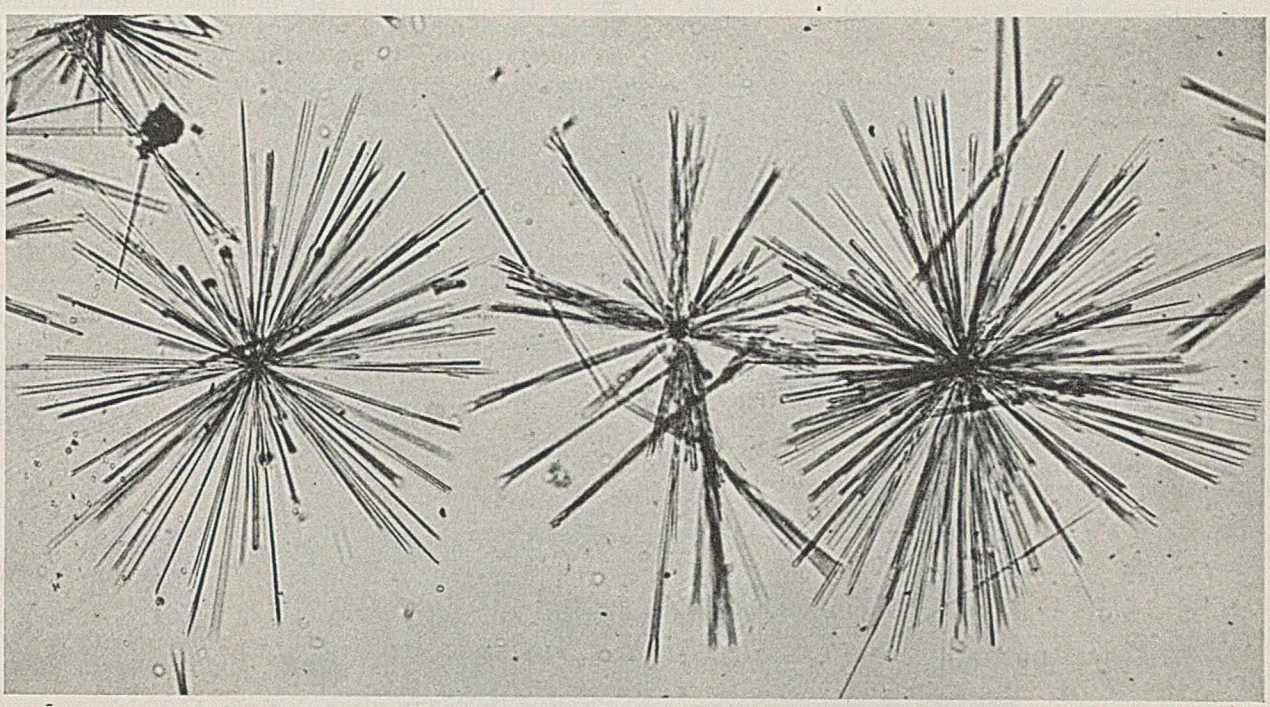


FIGURE 3. CRYSTALS OF $CaSO_4.2H_2O$

120 times actual size

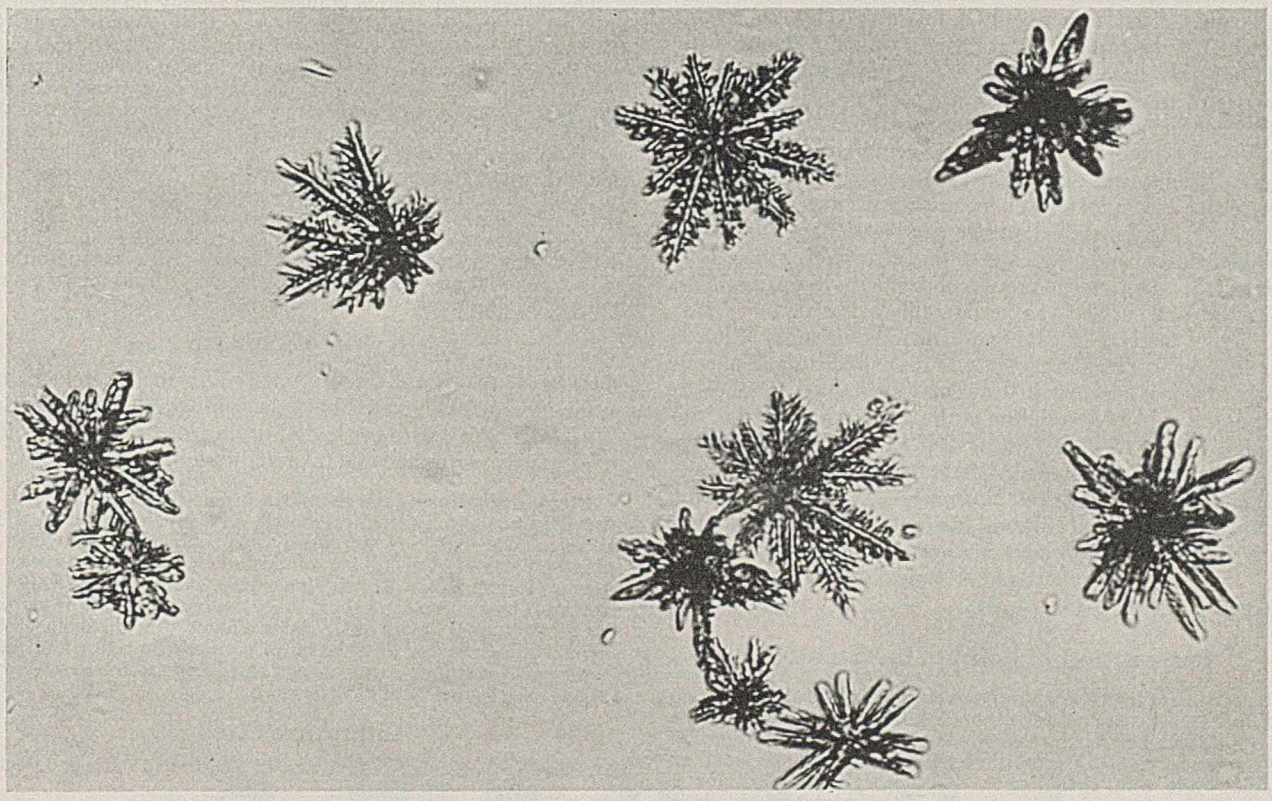


FIGURE 4. CRYSTALS OF $NH_4MgPO_4.6H_2O$

250 times actual size

TABLE I. TYPICAL MICROQUALITATIVE ANALYSES OF ALKALINE EARTH GROUP

	Barium Present	Barium Found	Strontium Present	Strontium Found	Calcium Present	Calcium Found	Magnesium Present	Magnesium Found
	Micrograms		*Micrograms*		*Micrograms*		*Micrograms*	
1	0	0	10	10	20	20	10	15
2	0	0	0	0	10	5	100	75
3	140	120	20	20	50	85	0	0
4	5	5	200	150	0	0	0	0
5	0	?	200	175	200	150	5	5
6	0	0	200	200	5	5	200	175
7	200	150	0	0	0	0	200	150
8	5	10	50	60	10	10	20	20

	Separation in Cone	Confirmatory Tests on Slide	
Limiting proportions	Ba:Sr = 1:1,000 (*5*) Sr:Ca = 1:500 (*5*) Sr:Mg = 1:500 (*5*) Ca:Mg = 1:300 (*5*)	Ba:Sr = 1:5 (Ba) Sr:Ba = 1:10 (Sr) Ca:Sr = 1:40 (Ca)	Ca:Ba = 1:40 (Ca) Mg:Ca = 1:50 (Mg) Ca:Mg = better than 1:50 (Ca)

monium phosphate hexahydrate. Centrifuge and compare the volume of precipitate with one of magnesium ammonium phosphate hexahydrate obtained from a known quantity of magnesium. Wash the precipitate once with 95 per cent ethyl alcohol.

CONFIRMATION OF MAGNESIUM. Dissolve precipitate P158 in such an amount of 6 *M* acetic acid as to form a solution containing 5 micrograms of magnesium per cubic millimeter. Place all or a portion of the solution on a slide and expose to fumes of ammonia. The presence of magnesium is confirmed by the slow crystallization of magnesium ammonium phosphate hexahydrate which, when seen through the microscope, has the appearance shown in the photomicrograph.

About forty solutions were analyzed in accordance with the above scheme and Table I shows eight representative results, as well as the limiting proportions of the main and confirmatory tests if made according to the scheme. In the case of the confirmatory tests, the elements in parentheses indicate those for which the tests are made. The limits of identification were: for separations, approximately 1 microgram; for confirmatory tests, barium and strontium 0.1 microgram, calcium 0.04 microgram (*2*), and magnesium 0.001 microgram (*2*).

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RECEIVED October 22, 1938.

Drying Etched Lead Surfaces

DONALD L. WOERNLEY, Buffalo Niagara Electric Corp., Buffalo, N. Y.

IN DEVELOPING a routine procedure for microscopically studying the crystalline structure of lead cable sheathing, difficulty was experienced in suitably drying the etched lead samples without formation of a film of oxide. It was found that an unoxidized surface could be maintained by submerging the etched lead sample in a 2 per cent solution of glacial acetic acid, but this necessitated the use of an accessory water-immersion lens. As a result a great portion of light reflected by the metallic surface was absorbed by the water and lenses; thus longer exposures were required when photographing and greater difficulty was experienced in focusing the camera.

In order to avoid the inconvenient water-immersion method, drying the etched sample in a blast of warm air after rinsing in alcohol and ether, and drying after rinsing in acetone were tested. During the process of drying by these methods, the etched lead surface was considerably oxidized.

A simple method of drying which minimizes oxidation is here described. This is part of a routine procedure used constantly in this laboratory in examining structure of lead cable sheathing. A brief description of the method long used in the Buffalo Niagara Electric Testing Laboratory for etching lead cable sheathing is also included, in order to afford a more complete picture of the entire etching process. The procedure for preparation of lead cable sheathing for microscopic examination is intended for lead containing small amounts of impurities. It is straightforward and will yield good results if care is taken.

Preparation of Sample for Etching

The section of lead sheathing to be studied is microtomed (*2*) on a Spencer Lens Company microtome No. 860, taking care that the lead is not scratched during this process. The angle of inclination of the microtome blade with the horizontal is made as small as possible while still obtaining a satisfactory cut. Sections 2 microns thick are sliced off at a time. If the microtome blade is feather-edged, it will easily be nicked and scratching of the lead will result.

Etching of Sample

A well-known solution (*1*) for etching is used, containing 15 ml. of glacial acetic acid, 20 ml. of concentrated nitric acid, and 80 ml. of water.

The microtomed sample is placed in freshly prepared etching solution, the temperature of the latter being approximately 42° C. The sample is frequently removed from the solution, rinsed in cold water, and swabbed. Frequent visual observation of the rinsed sample will show how the etching is progressing. In the final stage, the crystals appear mirrorlike when viewed with the naked eye. When the etching process is near the desired stage, the specimen is observed through the microscope to ascertain whether or not further etching is necessary. During etching the microtomed surface must not be exposed to air, but should be kept covered with a film of water when out of the etching solution. Since the thickness of the worked layer of lead resulting from microtoming is small, the etching process will consume only a short time, and any deep scratching resulting from microtoming will not disappear with the worked layer.

Drying of Sample

If the sample is not dried properly after being etched, the surface will quickly oxidize before a photograph can be taken. The following is a sure method of drying lead etchings, which is very easily performed and takes only a minute or two:

After quickly rinsing the sample in tap and distilled water, it is placed in a 130-ml. (4-ounce) bottle and covered with U. S. P. acetone. The bottle is stoppered with rubber through which is thrust a piece of glass tubing connected by means of a piece of pressure tubing to a water suction pump. The acetone vapor is drawn out by suction for a short time, the bottle is then inverted, and the acetone is drawn out. After a short time all the acetone vapor will be removed and the sample will become dry and may then be photographed in air. Lead surfaces dried in this way will remain intact for several days or more.

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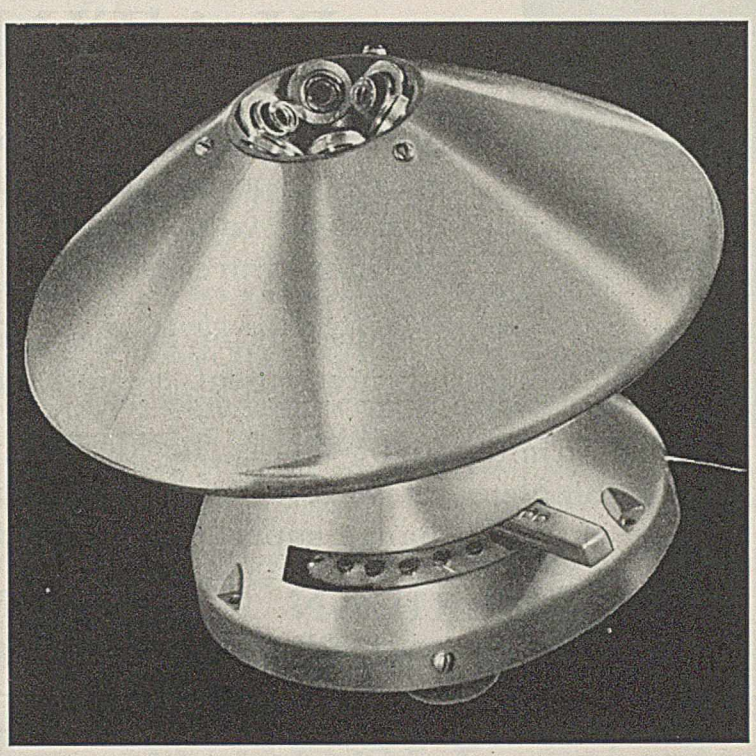
RECEIVED April 23, 1938.

MICRO—SEMI MICRO CENTRIFUGE



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