ANALYTICAL CHEMISTRY

Hallett Appointed Editor; Gibbs, Managing Editor

APPOINTMENT of Lawrence T. Hallett as editor of ANALYTICAL CHEMISTRY and of Robert G. Gibbs as managing editor was announced in the June 11 issue of *Chemical and Engineering News* by Editorial Director Walter J. Murphy.

These selections, plus those of editors for the three other applied journals of the AMERICAN CHEMICAL SOCIETY, are in accord with a policy of having editors for each of the applied journals: *Chemical and Engineering News, Industrial and Engineering Chemistry,* and *Journal of Agricultural and Food Chemistry.* These changes were effective June 15.

These promotions, made from within the staff, have been in anticipation of still further growth in the ACS publication program and in the service these publications render the profession and the chemical process industries.

Dr. Hallett first became connected with the journal in 1946, with the title of associate editor. In 1953, his title was changed to that of science editor. He has been the author of the monthly feature, the Analyst's Column, for several years.

Dr. Hallett did his undergraduate work at the University of British Columbia, receiving his bachelor's degree in 1923. He received his doctorate from the University of Wisconsin in 1928. His industrial experience has largely been with Eastman Kodak and General Aniline & Film in such fields as research, analytical, and microanalytical chemistry. His background also includes several years of college teaching of analytical chemistry. Dr. Hallett has been closely associated with the Division of Analytical Chemistry since its inception in 1937 as the Microchemical Section and later the Division of Analytical and Micro Chemistry. He was chairman of the division in 1938–39.

Robert G. Gibbs, a graduate of Clark University, joined the Washington editorial staff in 1948 as an associate editor in charge of the Washington News Bureau. In addition to his new duties with ANALYTICAL CHEMISTRY, he will continue to handle special assignments for the editorial director and executive editor of the applied journals.

ANALYTICAL CHEMISTRY, like all the other applied journals of the AMERICAN CHEMICAL SOCIETY, has grown considerably since its establishment in 1929, as the ANALYTICAL EDITION of INDUSTRIAL AND ENGINEERING CHEMISTRY. In the beginning it was issued on a quarterly basis; in 1933, bimonthly, and in 1937 it became a monthly edition of *Industrial and Engineering Chemistry.* In 1947, the Board of Directors accepted the recommendation of the editor that the name of the publication be changed to ANALYTICAL CHEMISTRY. At the same time, separate subscription lists were inaugurated for *I&EC* and ANALYTICAL CHEMISTRY. For many years, subscribers received both the journals.

The present ANALYTICAL CHEMISTRY bears little resemblance to the early quarterly editions, officially designated as the ANALYTICAL EDITION OF I&EC. The first quarterly issue, for example, contained 56 text pages, while today the average monthly issue of ANALYTICAL CHEMISTRY contains approximately 200 pages.

The journal has had phenomenal growth in recent years in comparison with the World War II period. In 1943, 788 editorial pages were printed; by 1955 this figure had almost tripled to 2259 pages. The journal has more than kept pace with a fast-moving segment of science and technology, one that has reached into both chemistry and physics and indeed, engineering, for basic knowledge and technological know-how.

The chemical process industries as they exist today would not be possible without modern physical-chemical analytical methods and techniques. Unquestionably what has gone on before, important as it has been, is but a prolog of what will happen in the broad field of analysis.

ANALYTICAL CHEMISTRY, nationally and internationally recognized for the services and leadership it has performed in the past, will expand its efforts in the future to serve the growing needs of analysts everywhere. The augmented staff will make it possible to improve still further the wide variety of services currently being provided for approximately 24,000 subscribers, a number which inevitably will increase materially as the chemical process industries continue to expand.

Walter J. Murphy *Editorial Director*

Instrumentation and Principles of Flame Spectrometry

Automatic Background Correction for Multichannel Flame Spectrometer

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An adaptation of a multichannel flame spectrometer to correct automatically for background radiation is described. Background intensity is measured to one or both sides of each line or band and is subtracted automatically from the line-plus-background intensity measured at the peak of the line or band. The performance of the instrument with this modification is discussed. Very high concentrations—above about 1 gram per liter—of some salts cause a depression of emission intensity in the flame. This depression can be eliminated by addition of ethyl alcohol to the solutions. A possible cause of this depression in emission intensity is discussed.

THE development of instruments for direct-reading analysis with a flame source has been largely restricted to those permitting the determination of only one element at a time—photometers or spectrophotometers. Instruments for the simultaneous determination of several elements in a sample using arc or spark sources—spectrometers—have been developed. Unfortunately, they are not well suited for use with flame sources. Earlier papers (*10, 11*) described a multichannel flame spectrometer for the simultaneous determination of several elements in one sample. This instrument was designed to take advantage of the stability of the flame source and the high intensity of emission by some elements in the hydrogen-oxygen flame. At present the instrument is being employed for the determination of sodium, potassium, magnesium, calcium, and strontium, and facilities for the determination of other elements can be added readily.

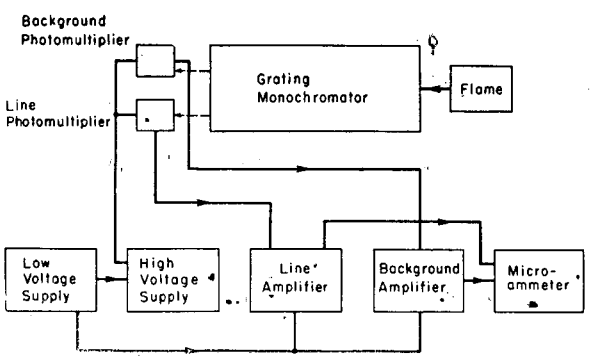

Figure 1. Block diagram of multichannel flame spectrometer modified for automatic background correction

When several elements in a single sample are to be determined simultaneously, it is necessary to consider any interactions between those elements which might affect their emission intensities in the flame. Several workers have reported that one cation affects the emission of light by another in the flame. For example, sodium and potassium have been variously reported to increase (*12*) and decrease (*1*) their respective emissions and that of calcium (*6, 9*). However, studies with the multichannel flame spectrometer (*8, 10*) indicate that the chief effect of extraneous cations is the production of heterochromatic background radiation. The effect on the monochromatic emission intensity of a given species measured above background is apparently negligible.

The background produced by each one of the extraneous elements in the sample is proportional to its concentration. Furthermore, all light intensities at a particular wave length, whether due to background or an emission line, are additive. It is possible, therefore, to estimate general flame background plus that due to extraneous ions and to subtract both from the line-plus-background reading, thus obtaining the line intensity. The sequence of calculations for such indirect corrections has been described and the precision of the method has been tested (*8*).

In the determination of the alkaline earths in biological fluids, the presence of relatively large amounts of sodium and potassium contributes background intensities which may be larger than the line emission intensities of the alkaline earths. Under these conditions the estimation of background due to sodium and potassium introduces an excessive error into the determination.

Direct measurement of background intensity has been found to give more precise results than the indirect estimation of background described above and previously. In addition, it is relatively easy to arrange the electrical connections in the multichannel flame spectrometer so that background is automatically subtracted from the line-plus-background readings. The automatic background correction is more rapid and more precise than the indirect method.

DESCRIPTION OF INSTRUMENT

Figure 1 shows a block diagram of the multichannel flame spectrometer modified for automatic background correction. The components—source, monochromator, power supplies, etc.—have been described in detail (*11*) and the analytical wave lengths are identical with those used previously (*8, 10, 11*). The instrument was modified by the addition of auxiliary exit slits and detectors to measure background, and pairs of amplifiers were connected to subtract background automatically.

An auxiliary exit slit of the same width as the main exit slit—i.e., 0.4 mm.— was placed 1/4 inch to one side of each line to be measured. As the linear reciprocal dispersion of the monochromator is 10.8 A. per mm., background is measured approximately 70 A. from the line. Scanning experiments (*8, 10*) have shown that the light intensity 1/4 inch from the line is almost entirely due to background.

The calcium oxide band head at 5498 A., used for the determination of calcium, is close to the strong sodium doublet at 5890 and 5896 A. The background intensity from sodium is higher on the long wave-length side of the calcium oxide band head than on the short wave-length side (*8*), while the background due to potassium is the same on either side of the band head. For this reason, the calcium channel is provided with two auxiliary exit slits, on either side of the band head. By an arrangement described below, the light passing through both of these exit slits is combined and the average intensity is measured.

Light passing through the main exit slit in each channel—line-plus-background—is received by a photomultiplier with its center placed 2 1/8 inches directly behind the exit slit. An auxiliary

photomultiplier is located alongside each of the line-plus-background detectors. As the diameter of a photomultiplier tube is 1 inch, the auxiliary photomultiplier is not directly behind the auxiliary exit slit. A front surface mirror, 1/2 inch behind the auxiliary exit slit, reflects the light slightly to one side, where it is received by the auxiliary photomultiplier. There is a mirror behind each of the axuiliary slits of the calcium channel, reflecting the light onto one photomultiplier tube.

The signal from each photomultiplier is sent to a separate direct current amplifier. Two amplifiers are used for each channel, one for line-plus-background and one for background alone. Each amplifier chassis also includes a high voltage power supply providing a regulated direct current voltage, variable from 700 to 900 volts, for the photomultiplier. The amplifier outputs are connected together in pairs. The "low" sides of the line-plus-background and background amplifiers are connected together; the "high" sides of the two amplifiers are connected across the microammeter. The meter thus indicates the difference between the two signals: the line intensity.

Figure 2 shows the instrument as adapted for automatic background correction. The inset shows the details of the arrangement of exit slits and photomultipliers.

The pairs of photomultipliers for measurement of line-plus-background and background are not critically matched. The photocells are chosen to have similar responses to the same light level; a difference of 10 to 15% is considered acceptable. Final matching of the photomultipliers is accomplished by a reduction of the dynode voltage on the more sensitive photomultiplier. For this purpose, background radiation is provided by the atomization into the flame of a solution containing one of the extraneous elements. With both amplifiers in the channel at their most sensitive settings, the dynode voltage on the more sensitive photomultiplier is reduced until the sensitivity of both detectors is equalized exactly. When this point is reached, closing of the entrance slit of the monochromator does not change the ammeter reading. In practice, the adjustment is made at the start of work and has remained stable throughout the day. The setting may be checked at any time by flushing a solution containing only extraneous cations through the burner.

The background photomultiplier on the calcium channel receives light from two exit slits, so that the total light intensity received by it is about twice the background intensity reaching the corresponding line-plus-background photomultiplier. When the dynode voltage to the background photomultiplier is adjusted in the usual way, the voltage is reduced so that the sensitivity of the detector is about one half of normal. In effect, the photocell averages the two light intensities.

EXPERIMENTAL

Concentrated stock solutions were prepared from reagent grade chemicals. These reagents had been examined previously by readings taken at the peaks of lines or bands and at wave lengths to either side (*8*), and found to be sufficiently free of impurities for this work. For example, reagent grade sodium chloride did not contain detectable amounts of calcium or magnesium. Metal chlorides were dried overnight or longer at 130° C., weighed on an analytical balance, and dissolved in water purified by passage through a mixed-bed ion exchange column. Appropriate aliquots of these stock solutions were diluted to prepare the solutions for the experiments described in the following section. All solutions to be compared directly were prepared at the same time.

The method of operation of the instrument has been described (*8, 11*). Only slight changes in procedure need be made when the automatic background correction is used. Matching of photocells by adjustment of the dynode voltages has been described above. Thereafter, readings may be obtained in the usual fashion, except that care must be taken to ensure that both the line-

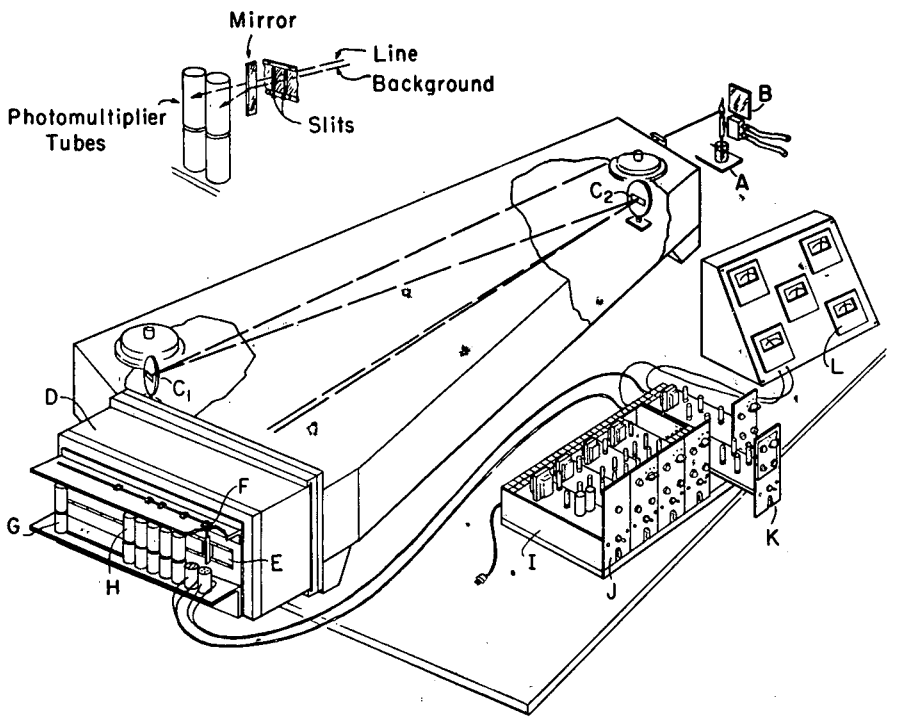

Figure 2. Multichannel flame spectrometer modified for automatic background correction

Inset shows details of exit slit–photomultiplier arrangement

A. Beckman atomizer burner
B. Plane front surface mirror
C_1. Collimating mirror
C_2. Grating
D. Detector mount
E. Aperture in detector mount
F. Adjustable holder for front surface mirror
G. Photomultiplier mount
H. Photomultiplier tube
I. Bank of amplifiers
J. Low voltage power supply
K. High voltage power supplies and amplifiers
L. Microammeters

plus-background and background amplifiers of a particular channel are at the same sensitivity setting at all times.

RESULTS

The performance of the background correction has been tested by the comparison of readings obtained with solutions that contain the cation to be measured alone and in the presence of considerably larger concentrations of extraneous cations. Many of these experiments have been performed on the calcium channel. The proximity of the strong sodium doublet to the calcium oxide band head introduces complications not present at the other analytical wave lengths, thus providing a test under the most difficult conditions.

Table I summarizes a set of data on the calcium channel for solutions containing various concentrations of calcium alone and in the presence of 1000 p.p.m. of sodium. The meter readings are averages of 17 consecutive determinations and the standard deviation of each set of determinations is given in microamperes and as a percentage of the average meter reading. The background from 1000 p.p.m. of sodium was 380 μa. at this wave length, approximately eight times the signal from 1 p.p.m. of calcium. The photomultipliers were matched by adjusting the dynode voltages when a solution containing 300 p.p.m. of sodium was flushed through the burner. The background from 300 p.p.m. of sodium was 125 μa.

Table II shows similar results for calcium in the presence of 1500 p.p.m. of sodium. The results given in this and all succeeding tables represent single determinations on each solution. The background from 1500 p.p.m. of sodium was 470 μa. At this level of background intensity, noise arising in the photomultiplier becomes a serious factor; the ammeter needle quivers rapidly, so that it is difficult to obtain readings.

Table I. Effect of 1000 P.P.M. of Sodium on Calibration Curve for Calcium

Ca, P.P.M.	Na, P.P.M.	Meter Reading,[a] μa.	Standard Deviation μa.	Standard Deviation %
1	0	48.3	1.5	3.1
3	0	147.7	1.6	1.1
10	0	511	8.8	1.7
1	1000[b]	46.9	1.4	2.9
3	1000	139.3	2.6	1.9
10	1000	494	7.3	1.5

[a] Average of 17 consecutive determinations.
[b] Background from 1000 p.p.m. of sodium = 380 μa.

Table II. Effect of 1500 P.P.M. of Sodium on Calibration Curve for Calcium

Ca, P.P.M.	Na, P.P.M.	Meter Reading, μa.	Ca, P.P.M.	Na, P.P.M.	Meter Reading, μa.
1	0	45	1	1500[a]	43
3	0	139	3	1500	136
10	0	500	10	1500	450
30	0	1390	30	1500	1300
100	0	4500	100	1500	4000

[a] Background from 1500 p.p.m. of sodium = 470 μa.

Table III gives the data obtained on the strontium channel for solutions containing 3 p.p.m. of strontium and various concentrations of sodium, potassium, and calcium. For this experiment, calcium chloride was prepared from Spec-Pure calcium carbonate (Johnson, Matthey and Co., Ltd., London, England). Reagent grade calcium chloride was found to contain a significant quantity of strontium, detectable with the flame spectrometer. Strontium was also found in the sample by spectrographic analysis with a spark source.

Table IV shows readings obtained on the calcium channel with solutions containing various concentrations of calcium alone and in the presence of 1000 p.p.m. of potassium. The solutions containing excess potassium gave definitely lower readings than those containing calcium only. The largest discrepancy was observed for the solutions containing 30 p.p.m. of calcium; the reading for the solution with excess potassium is lower by 19% than the reading for the solution containing calcium alone. A similar tendency may be noticed in Table II, where an excess concentration of sodium chloride caused a slight depression of the readings.

The low readings for calcium obtained in the presence of 1000 p.p.m. of potassium could be caused by either failure of the background correction or a decrease in emission intensity in the flame.

Table III. Effect of Extraneous Ions on Readings of Strontium Channel

Sr, P.P.M.	Na, P.P.M.	Ca, P.P.M.	K, P.P.M.	Meter Reading, μa.
3	10	0	0	43
3	100	0	0	44
3	1000[a]	0	0	48
3	0	10	0	45
3	0	100	0	45
3	0	1000[b]	0	45
3	0	0	10	46
3	0	0	100	47
3	0	0	1000[c]	47

[a] Background from 1000 p.p.m. of sodium = 180 μa.
[b] Background from 1000 p.p.m. of calcium = 90 μa.
[c] Background from 1000 p.p.m. of potassium = 155 μa.

In order to distinguish between these two possible causes, other metals were substituted for potassium in the solutions; 1000 p.p.m. of lithium, as the chloride, depressed the readings from 1 to 100 p.p.m. of calcium by an average of 12%. The readings from the same concentrations of calcium were decreased by an average of 10% when 2.5 grams per liter of cadmium chloride were added to the solutions. The background at the calcium oxide band head from this concentration of cadmium chloride was only 1 μa. Zinc chloride, at a concentration of 2.5 grams per liter, reduced the emission of 1 to 100 p.p.m. of calcium by an average of 16%. Ammonium chloride, at concentrations as high as 2.6 grams per liter, had no detectable effect on the readings obtained on the calcium channel.

The depression in emission intensity caused by the presence of such large amounts of some salts can be alleviated by the addition of ethyl alcohol to the solutions. As an example, Table V shows the readings obtained for 1 to 100 p.p.m. of calcium alone and in the presence of 2000 p.p.m. of potassium. All of the solutions contained 25% of ethyl alcohol by volume. In spite of the large amount of extraneous salt added, good agreement was obtained between the two sets of solutions.

Table VI lists the readings obtained with solutions containing various concentrations of strontium alone and with 1000 and 2000 p.p.m. of sodium added. The readings obtained for the solutions containing 2000 p.p.m. of sodium show the depression in emission intensity caused by the presence of a large amount of extraneous salt.

Similar results have been obtained for 1 to 100 p.p.m. of strontium alone and with 1000 p.p.m. of potassium added. For example, 1 p.p.m. of strontium alone gave a reading of 25 μa. and 1 p.p.m. of strontium with 1000 p.p.m. of potassium gave a reading of 23 μa.

DISCUSSION

The results obtained with the automatic background correction were much more precise than those obtained by corrections based on indirect estimation of background. (See Figure 6 of *8*.) In Table II, for example, the background intensity from 1500

p.p.m. of sodium (470 μa.) was more than ten times the line intensity from 1 p.p.m. of calcium. Even under these extreme conditions, the difference in ammeter readings for the two solutions containing 1 p.p.m. of calcium was only 2 μa. This represents an error in the determination of 5% of the amount present, while Figure 6 of (*8*) indicates that errors as large as 100% may be expected for this ratio of background and line intensities when the indirect method of background correction is employed.

The ratios of background and line intensities are not as large in the other results shown; but most involve background intensities considerably higher than the line intensities. Under these conditions, a comparatively small error in the background correction would be reflected in a much larger difference in the ammeter readings. In the example cited above, a difference of 2 μa. in the ammeter reading represents a 5% error in the determination, but only a 0.43% error in the background correction. In general, the background correction appears to have a precision within ±1%.

Noise originating in the photomultiplier tubes rises with increasing light intensity. At the higher levels of background intensity this noise becomes a serious source of error, causing the microammeter needle to waver rapidly, so that readings are obtained with difficulty. This source of error might be eliminated readily by modification of the amplifiers to provide a 1- or 2-second response time.

The automatic background correction appears to be capable of dealing with any levels of background intensity encountered in routine analyses. A 10 to 1 ratio of background to line intensities was reached only when a 1500-fold excess of sodium was present in the determination of calcium. Such large amounts of extraneous salts appear to cause a depression of emission in the flame, introducing a source of error distinct from background.

The cause of the decrease in emission intensity when relatively high concentrations—above about 1 gram per liter—of extraneous salts are present has not been investigated in detail. It has been shown (*8*) that the formation of compounds which do not evaporate during their passage through the flame can bring about a decrease in emission intensity. It is considered likely that the presence of high concentrations of certain salts in the flame causes the formation of large particles in the flame after evaporation of the solvent from the spray. These particles are too large to evaporate completely during the relatively brief period of their passage through the flame. Each particle may include small amounts of the element to be determined, reducing the amount of that species accessible to excitation. Ammonium chloride decomposes at a lower temperature than the other salts tested, so that it evaporates at a rate similar to that of the solvent and does not interfere with evaporation of the solid particles remaining. Ethyl alcohol may lower the surface tension of the solutions, reducing the particle size in the spray.

Other background correction methods than the one described in this paper should be feasible. It should be possible, for example, to use the same exit slit and detector to measure line-plus-background and background intensities alternately either by rapidly shifting the spectrum at the focal plane or by moving the exit slit and detector along the focal plane. An alternating current amplifier could be used which would respond to the alternating part of the signal—the line intensity. Either of these methods would permit the use of only one amplifier per channel, at the expense of some mechanical complication.

The use of separate exit slits and detectors for line-plus-background and background was conditioned by the design of the instrument before background correction was introduced. Since no major changes in the monochromator or in the amplifiers were necessary, it was possible to make the modification for automatic background correction quickly and easily. Unfortunately, there does not seem to be any such simple way of adapting existing commercial flame photometers for automatic background correction, though several workers have recently reported good results in making background corrections on the Beckman Model DU spectrophotometer with the flame attachment by direct estimation (*2–5*). The background is read to one side of the line or band. This value is subtracted from the line-plus-background intensity measured at the peak of the line or band. Although this method can give good results, it requires two readings for each determination and is therefore much slower than the automatic background correction described in this paper.

Table IV. Effect of 1000 P.P.M. of Potassium on Calibration Curve for Calcium in Aqueous Solutions

Ca, P.P.M.	K, P.P.M.	Meter Reading, μa.	Ca, P.P.M.	K, P.P.M.	Meter Reading, μa.
1	0	43	1	1000	40
3	0	136	3	1000	125
10	0	460	10	1000	400
30	0	1360	30	1000	1110
100	0	4300	100	1000	4300

Table V. Effect of 2000 P.P.M. of Potassium on Calibration Curve for Calcium

(All solutions contained 25% ethyl alcohol by volume)

Ca, P.P.M.	K, P.P.M.	Meter Reading, μa.	Ca, P.P.M.	K, P.P.M.	Meter Reading, μa.
1	0	81	1	2000	80
3	0	173	3	2000	189
10	0	560	10	2000	630
30	0	1630	30	2000	1610
100	0	5200	100	2000	5100

Table VI. Effect of 1000 and 2000 P.P.M. of Sodium on Calibration Curve for Strontium

Sr, P.P.M.	Na, P.P.M.	Meter Reading, μa.	Sr, P.P.M.	Na, P.P.M.	Meter Reading, μa.	Sr, P.P.M.	Na, P.P.M.	Meter Reading, μa.
2	0	39	2	1000[a]	40	2	2000[b]	35
6	0	128	6	1000	132	6	2000	119
20	0	420	20	1000	410	20	2000	400
60	0	980	60	1000	980	60	2000	990

[a] Background from 1000 p.p.m. of sodium = 127 μa.
[b] Background from 2000 p.p.m. of sodium = 260 μa.

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RECEIVED for review November 8, 1955. Accepted April 30, 1956. Preliminary accounts presented at Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 2, 1955, and meeting of Optical Society of America, April 8, 1955 (*7*). Studies supported by grant-in-aid from the Rockefeller Foundation, New York.

Flame Spectrophotometric Determination of Microgram Quantities of Copper

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No detailed procedures have previously been published for the rapid, precise, and convenient determination of micro quantities of copper. Flame spectrophotometric studies at the copper line (324.7 mμ) showed that the radiant power of copper was greatly enhanced by aspirating from an 80% methanol solvent. Interference studies indicated that nitric acid could be tolerated in appreciable quantities. Most cations show considerable radiation interference; however, the increase in the radiant power of copper caused by the addition of nine cations to standard copper solutions was determinable at a wave length of 325.1 mμ. Inasmuch as no preliminary separations are necessary, the method is adaptable to routine determinations. The results are as accurate as conventional microchemical procedures.

ALTHOUGH there are many publications on the determination of alkali and alkaline earth metals by flame photometry, relatively few investigators have reported on the flame photometric determination of copper (*1*, *3*, *9–11*, *15–17*). Of these, only Dean (*3*) and Jordan (*11*) have described methods in detail. Dean (*3*) worked with nonferrous alloys containing copper in the range from 0.3 to 2.5%, and Jordan (*11*) analyzed for small amounts of copper in a hydrochloric acid extract of gasoline. Work by Dean and Lady (*5*, *13*) indicates that copper can be determined in micro quantities by flame photometry after a preliminary extraction of the copper as the salicylaldoxime with either chloroform or amyl acetate.

Table I. Radiant Power of 4 γ per Ml. of Copper with Various Alcohols as Solvent

Alcohol	%	Radiant Power, Scale Divisions[a] Cu + ROH	ROH	Net Cu
Methanol	95	54.2	21.5	32.7
	80	51.2	26.0	25.2
	40	42.5	26.5	16.0
	20	38.3	23.4	14.9
Ethyl alcohol	95	53.8	26.1	27.7
	80	47.7	29.0	18.7
	40	41.8	28.2	13.6
	20	40.6	24.2	16.4
1-Propano	95	49.8	28.4	21.4
	80	45.4	30.4	15.0
	40	42.3	29.0	13.3
	20	40.2	25.8	14.4
2-Propanol	95	50.7	30.4	20.3
	80	46.0	30.1	15.9
	40	42.2	30.4	11.8
	20	41.4	25.5	15.9
2-Methyl-2-propanol	95	45.9	34.0	11.9
	80	43.7	34.0	9.7
	40	42.4	29.7	12.7
	20	42.3	25.8	16.5

[a] Instrument adjusted to read 100 on the transmittance scale with 12 γ per ml. of copper in 80% MeOH.

This study describes a rapid procedure for the determination of small concentrations of copper (1 to 8 γ per ml.) and presents a simple technique for reducing the error caused by the presence of varying amounts of interfering cations. Although the method was developed principally to determine micro quantities of copper in beverage alcohol, the procedure is applicable to the determination of copper in complex solutions containing many inorganic components.

APPARATUS AND CHEMICALS

Emission measurements were made with a Beckman Model DU spectrophotometer equipped with a Model 4300 photomultiplier accessory and Model 9200 flame photometry attachment. A Model 4020 atomizer-burner utilizing an oxyhydrogen mixture was the source of excitation.

Analytical grade chemicals were used throughout the investigation. Stock solutions of copper, iron, aluminum, and magnesium ions were prepared by reacting each metal with a minimum of concentrated nitric acid and then diluting to volume with double-distilled water. Solutions of sodium, potassium, manganese, nickel, and lead ions were prepared from their nitrates, and a solution of calcium ions was prepared by reacting calcium carbonate with concentrated nitric acid. The solutions were stored in polyethylene containers.

A composite stock solution containing nine cations was used to study interference phenomena. This solution was prepared by pipetting into a 500-ml. volumetric flask 20 ml. of 0.5% aluminum, 150 ml. of 0.5% iron, 100 ml. of 0.5% magnesium, 50 ml. of 0.5% calcium, 5 ml. of 12.5% sodium, 10 ml. of 12.5% potassium, 1 ml. of 15.35% manganese, 100 ml. of 0.125% nickel, and 10 ml. of 0.5% lead. The flask was filled to the mark with double-distilled water and thoroughly agitated. This composite stock solution contained 1.5 γ per ml. of copper as determined by the technique described below.

EXPERIMENTAL PROCEDURE

Preliminary experiments with pure solutions of copper nitrate showed that the radiant power of copper was greatest at a wave length of 324.7 mμ with a slit width of 0.03 mm. This was also reported by Dean (*3*). The following Beckman DU spectrophotometer settings were used throughout the investigation:

Wave length	
Copper line	324.7 mμ
Flame background	325.1 mμ
Selector	0.1
Sensitivity on photomultiplier battery box	Full, 60 volts per dynode
Resistor	22 megohms
Slit width	0.03 mm.
Hydrogen	2.5 lb. per sq. in.
Oxygen	10 lb. per sq. in.

Pressures of 1.5 and 12 pounds per square inch for hydrogen and oxygen, respectively, were also satisfactory. The wave length setting must be exact in order to obtain the maximum energy response and to reproduce the standard curve. The following procedure was adopted to minimize instrumental errors.

Make the instrument settings indicated above, light the flame, close the shutter, and zero the null meter by adjusting the dark current control. Set the transmittance dial to read 100% transmittance, aspirate a copper solution containing 12 γ per ml. and zero the null meter by means of the sensitivity control. Close the shutter and, if necessary, readjust the dark current control to zero the meter. Aspirate the copper solution that is to be determined, open the shutter, and turn the transmittance dial to zero the null meter; close the shutter and record the radiant power from the transmittance scale. Repeat this procedure at least twice. Each radiant power value reported is the average of three readings varying by not more than ±0.5 scale division taken directly from the transmittance scale.

RESULTS

Effect of Alcohols. Numerous investigators have studied the effect of organic solvents on the flame photometric emission of certain elements. Curtis, Knauer, and Hunter (*2*) showed that

Table II. Radiant Power of Copper in 80% Methanol Containing Various Acids

Acid	Cu Concn., γ per Ml.	Radiant Power, Scale Divisions[a] Cu + Acid	Acid	Net Cu
0.04*M* H_3PO_4	4	55.5	30.3	25.2
0.1*M* H_3PO_4	4	60.5	34.7	25.8
2.0*M* H_3PO_4	4	108.7	99.7[b]	9.0
0.05*M* HNO_3	8	76.7	26.2	50.5
1.0*M* HNO_3	8	77.4	26.8	50.6
2.0*M* HNO_3	8	81.9	26.2	55.7
0.05*M* HOAc	8	76.7	26.3	50.4
1.0*M* HOAc	8	81.5	26.0	55.5
2.0*M* HOAc	8	86.1	23.9	62.2
0.05*M* HCl	8	75.9	26.3	49.6
1.0*M* HCl	8	70.7	27.5	43.2
1.5*M* HCl	8	68.2	27.7	40.5
0.05*M* H_2SO_4	8	74.7	26.8	47.9
1.0*M* H_2SO_4	8	72.7	31.1	41.6
2.0*M* H_2SO_4	8	72.2	34.5	37.7

[a] Instrument set to read 100 on transmittance scale with 12 γ per ml. of copper in 80% MeOH; 4 and 8 γ per ml. of copper in 80% MeOH gave net radiant powers of 25.5 and 50.5 scale divisions, respectively. [b] Instrument set to read 60 on transmittance scale with 12 γ per ml. of copper in 80% MeOH.

greater radiant power can be obtained by atomizing from hydrocarbons and ethers than by atomizing from aqueous solutions. They studied barium, calcium, and organosodium compounds. Dean and Thompson (*6*) studied the relative radiant power of boron from methanol-water solutions. Kingsley and Schaffert (*12*) reported on the flame photometric determination of sodium, potassium, and calcium in various organic solvents including alcohols, and found that acetone produced the greatest enhancement of radiant power. Dean and Lady (*4*) used 2,4-pentanedione as the extracting reagent and combustible solvent for the determination of iron. Fink (*8*) investigated the use of alcoholic solutions for the flame photometric determination of calcium and magnesium and showed that pentyl alcohol produced a threefold increase in sensitivity. A very recent general study (*13*) of the solvent effects on flame emissions has been reported. Unfortunately, this report was called to the authors' attention at the conclusion of this work.

Preliminary experiments indicated that 95% ethyl alcohol by volume increased the radiant power of copper. Consequently, different alcohols were tested at varying concentrations to determine their effect on the radiant power of 4 γ per ml. of copper. The data in Table I show that high concentrations of methanol produce the greatest net emissions. It was decided, therefore, to use 80% methanol as a solvent because this concentration contains sufficient water to dissolve large amounts of salts.

The calibration curves for copper in 80% methanol and in water are linear. A comparison of the curves reveals that 0 to 12 γ per ml. of copper in 80% methanol gives a spread from 25 to 100 scale divisions, representing a total of 75 scale divisions with each division equivalent to 0.16 γ per ml. of copper. When water is the solvent and the same sensitivity is used, the spread is from 18 to 49 scale divisions, or a net of 31 scale divisions with each division equivalent to 0.40 γ per ml. of copper. Therefore, an error of 1 scale division represents less error, in terms of copper, when 80% methanol is the solvent than when water is the solvent.

A comparison of the curves obtained by aspirating 0 to 100 γ per ml. of copper in water and in 80% methanol at identical sensitivities was noteworthy. Dean (*3*) obtained almost the complete spread of the transmittance scale when burning 0 to 100 γ per ml. of copper in water. With 80% methanol as the solvent the same spread was obtained with 0 to 28 γ per ml.

Effect of Anions. The effect of various anions was investigated because acids are generally used in the analysis of metals. Table II illustrates the data obtained when acids are present in 80% methanol containing 4 and 8 γ per ml. of copper. A peculiar effect is encountered when phosphoric acid is present. The addition of either 0.04*M* or 0.1*M* phosphoric acid increases the background emission without affecting the net emission by copper. Phosphoric acid, 2*M*, exerts a pronounced inhibiting action on the radiant power of copper. This phenomenon was observed by Parks, Johnson, and Lykken (*14*), who found that phosphate had an inhibiting effect on the radiant power of sodium and potassium. Dippel, Bricker, and Furman (*7*) demonstrated that phosphoric acid shows a continuous emission between 320 and 700 mμ.

Nitric acid was tolerated in appreciable quantities. Concentrations of 0.05*M* and 1*M* had no effect on the radiant power of copper, whereas a 2*M* concentration showed an increase in the radiant power of copper corresponding to 5 scale divisions. The radiant power of copper is definitely enhanced by 1*M* and 2*M* acetic acid in 80% methanol.

Hydrochloric and sulfuric acids caused the radiant power of copper to decrease as the concentration of these acids was increased.

Effect of Cations. Various metal ions in concentrations of 50 and 200 γ per ml. were added individually to 4 γ per ml. of copper in 80% methanol to ascertain the extent to which these ions contribute radiation interferences. The data presented in Table III show that both concentrations of aluminum, iron, magnesium, calcium, lead, potassium, and nickel cations repress the radiant power of copper while the manganese ion appears to exert a slight enhancement. The addition of 50 γ per ml. of lithium ion had no effect, while 200 γ per ml. of lithium ion and 50 and 200 γ per ml. of sodium ions increased the background emission slightly without affecting the net copper emission. Dean (*5*). in his work with aqueous solutions, found that large quantities of aluminum, lead, magnesium, manganese, iron, nickel, and potassium ions did not contribute radiation interferences.

Table III. Effect of Cations on Radiant Power of 4 γ per Ml. of Copper in 80% Methanol[a]

Cation Added	γ per Ml.	Radiant Power, Scale Divisions Cu + cation	Cation	Net Cu
Al^{+++}	50	38.8	25.1	13.7
Al^{+++}	200	39.9	25.0	14.9
Ca^{++}	50	39.0	25.5	13.5
Ca^{++}	200	39.9	27.2	12.7
Fe^{+++}	50	39.0	25.1	13.9
Fe^{+++}	200	40.2	26.9	13.3
K^{+}	50	39.5	25.0	14.5
K^{+}	200	38.8	25.2	13.6
Li^{+}	50	51.5	25.8	25.7
Li^{+}	200	54.6	28.0	26.6
Mg^{++}	50	38.5	25.2	13.3
Mg^{++}	200	39.9	25.5	14.4
Mn^{++}	50	52.3	24.5	27.8
Mn^{++}	200	52.6	25.3	27.3
Na^{+}	50	54.2	28.2	26.0
Na^{+}	200	55.7	29.7	26.0
Ni^{++}	50	38.7	25.2	13.5
Ni^{++}	200	38.6	26.1	12.5
Pb^{++}	50	38.1	24.8	13.3
Pb^{++}	200	38.3	26.0	12.3

[a] 4 γ per ml. of copper gave a net radiant power of 25.5 scale divisions.

Experiments were than performed to determine the extent of radiation interference caused by the addition of a combination of nine cations to standard copper solutions, and the degree to which this interference could be corrected. Aqueous and 80% methanol solutions were prepared containing known amounts of copper and nine cations in varying concentrations. The instrument was adjusted to read 100% transmittance when aspirating 12 γ per ml. of copper and the standard curve was checked with solutions containing 1, 4, and 8 γ per ml. of copper. Actual emission readings were then taken on each solution containing the copper with added cations.

In order to correct for the radiation caused by the interfering cations, the following technique was employed. Adjust the instrument to read 100% transmittance while aspirating 12 γ per ml. of copper and then move the transmittance dial to the radiant power (T_s) obtained by aspirating the solvent alone. (These values were 25 and 39 scale divisions for 80% methanol and water, respectively.) Rotate the wave length dial very slowly

Table IV. Effect of Cation Mixture on Radiant Power of Copper

Composite Stock Soln. Added, % (v./v.)	Copper, γ per Ml. Present	Found
5[a]	8.0	7.9
	6.0	6.0
	4.0	4.0
	2.0	1.9
	1.0	0.9
10[a]	8.0	8.0
	6.0	6.0
	4.0	4.1
	2.0	2.0
	1.0	0.9
15[a]	8.0	8.0
	6.0	6.0
	4.0	3.9
	2.0	1.9
	1.0	1.1
10[b]	8.0	7.9
	6.0	6.0
	4.0	3.9
	2.0	1.9
	1.0	0.9
15[b]	8.0	8.0
	6.0	6.1
	4.0	4.1
	2.0	1.9
	1.0	0.9
40[b]	8.0	8.0
	6.0	6.0
	4.0	4.0
	2.0	2.0
	1.0	1.0

[a] 80% methanol solvent.
[b] Water solvent.

until the null point is obtained on the meter. With this instrument the wave length was found to be 325.1 mμ. Measure the interfering radiation of each copper solution containing the interfering cations. Calculate the radiant power due to copper by the following equation:

$$T_c = T_{324.7} - (T_{325.1} - T_s)$$

where
T_c = radiant power due only to copper
T_s = background of pure solvent

Read the copper equivalent of T_c from the standard curve. Subtract the amount of copper occurring as a contaminant in the composite stock solution to obtain the amount of copper recovered.

Table IV illustrates the data obtained by utilizing this technique. It is apparent that radiation interferences produced by the addition of large concentrations of foreign ions can be corrected by determining the excess emission at a wave length of 325.1 mμ. The results obtained with 80% methanol as solvent were comparable to those obtained with water.

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RECEIVED for review October 19, 1955. Accepted April 16, 1956.

Differential Spectrophotometric Method for Determination of Uranium

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A rapid spectrophotometric method is described for the determination of uranium in high concentrations with a precision equal to that of conventional volumetric or gravimetric methods. The relative absorbance of uranyl ion is measured at a wave length of 418 mμ against a highly absorbing reference standard. From the difference in absorbance, the concentration of uranyl ion in excess of that in the reference standard solution is determined with a high degree of precision. A method for the determination of the optimum concentration of the reference standard is presented. The effects of trace impurities are evaluated. In the optimum range of 20 to 60 mg. of uranium per ml., the precision is within 0.3%.

A LARGE number of methods have been described (*9*) for the gravimetric and volumetric determination of macro quantities of uranium. Several spectrophotometric methods, dependent on colors developed by the addition of chromogenic reagents, have been applied to the determination of uranium, particularly in low concentrations. However, few procedures for uranium determination, based on the color of the uranyl ion, have been published. A colorimetric method was used by Scott and Dixon (*11*) for the determination of uranium in leach liquor. Rodden (*10*) noted that a differential spectrophotometric technique was used by Brackenbury for the estimation of uranium in alkali peroxide solutions. Recently, a method in which uranium is determined spectrophotometrically in perchloric acid was reported (*8*). While the present paper was being reviewed, Bacon and Milner of the British Atomic Energy Research Establishment reported the results of a similar study (*1, 2*). They make use of differential spectrophotometry for the determination of uranium in the metal, binary and tertiary uranium-base alloys, and uranium oxide.

Ordinary spectrophotometric methods dependent on the absorbance of uranyl ions fail to yield satisfactory precision for macro amounts of uranium, because the absorbance scale must

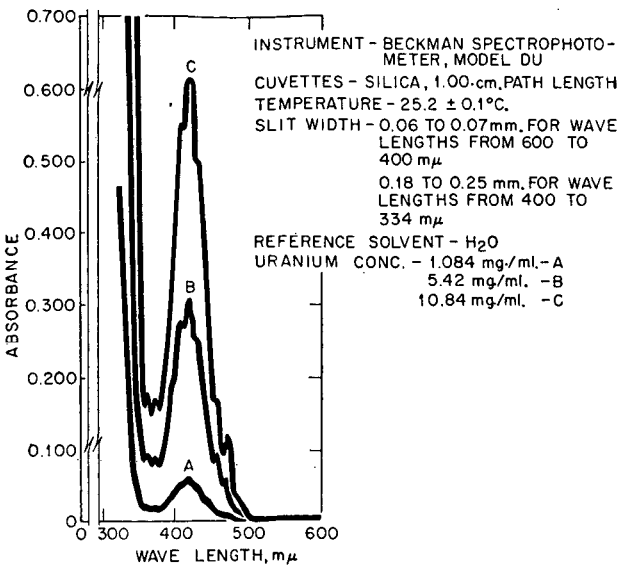

Figure 1. Absorbance spectrum of uranyl sulfate

cover too wide a concentration range. To circumvent this difficulty, a differential spectrophotometric procedure has been applied to the estimation of uranium in uranyl sulfate solutions.

By the use of the differential spectrophotometric techniques which involve measurement of relative rather than absolute concentrations, the precision may be considerably improved. In fact, the results may often be made to equal the precision attained by classical gravimetric or volumetric methods. Several applications of differential spectrophotometry and the theoretical aspects of this technique have been discussed by Hiskey (*6*, *7*) and Bastian (*3*, *5*).

The development of the differential spectrophotometric method for uranium required a study of the absorption spectrum of uranyl sulfate, the selection of suitable reference standards, and the effect of trace amounts of diverse ions and excess acid. These studies resulted in the development of a procedure which makes possible the rapid and reliable estimation of uranium in concentrations of 20 to 60 mg. per ml. in the final solution.

ABSORPTION SPECTRUM OF URANYL SULFATE SOLUTIONS

Figure 1 represents the absorption spectrum of uranyl sulfate solutions over the wave length range, 320 to 550 mμ. Uranyl and sulfate were determined by gravimetric methods in a stock solution of uranyl sulfate used for the preparation of the four test solutions. The ratio of the two ions was thus determined to be 1 to 1.

The Beckman Model DU spectrophotometer used in making the measurements was equipped with a tungsten lamp for the visible spectrum. A liquid-cooled mounting block and thermospacer (Beckman attachments Nos. 2360 and 2021) were used to maintain the temperature of the sample and reference solutions constant at 25 ± 0.1° C. The sensitivity setting was kept constant throughout the series of measurements. To accomplish this, the galvanometer zero adjustment was made by varying the width of slit. The wave length scale was calibrated by means of the lines of a mercury arc source.

The absorbance of uranyl sulfate was found to be a maximum at a wave length of 418 mμ. All measurements were, therefore, taken at this peak.

SELECTION OF REFERENCE STANDARDS

A method described by Hiskey (*7*) was followed in establishing the optimum concentration of reference solution to be used in the determination of uranium by the differential spectrophotometric method. Hiskey demonstrated that the relative error of the method, dc_2/c_2, and the concentration of the standard reference solution, c_1, are related as indicated by Equation 1:

$$\frac{dc_2}{c_2} = 0.43\, d \left(\frac{I_2}{I_1}\right) \times \frac{1}{\frac{I_2}{I_1}\left(\log \frac{I_2}{I_1} - a'c_1\right)} \qquad (1)$$

when

c_2 = concentration of the more concentrated solution (unknown)

c_1 = concentration of the more dilute solution (reference standard)

$\frac{I_2}{I_1}$ = ratio of transmittance of the more concentrated solution to that of the reference solution

a' = absorbance coefficient for a point on the absorbance-concentration graph for cell of 1-cm. light path

$\frac{dc_2}{c_2}$ = relative error of method

$\frac{d(I_2/I_1)}{I_2/I_1}$ = relative instrument error

For dilute solutions, the absorbance of which conforms to Beer's law, the absorbance coefficient is, of course, independent of the concentration. For more concentrated solutions, the absorbance-concentration graph becomes nonlinear and the absorbance coefficient decreases with increasing concentration. For cells of 1-cm. light path the absorbance coefficient, a', for a point on the nonlinear portion of the curve equals the slope of a tangent to that point. In applying Equation 1, it is assumed that a' is constant over the concentration range c_1 to c_2. No serious error is introduced by this assumption, so long as Δc is kept small.

If the concentrations of the standard reference solution, c_1, and of the unknown, c_2, are nearly equal, I_2/I_1 will very nearly equal unity, and $\log I_2/I_1$ will be practically zero. Under these conditions, for practical purposes, $dc_2/c_2 \propto -1/a'c_1$. From this, it is apparent that the relative error of the method, dc_2/c_2, will be a minimum when $a'c_1$ is a maximum. The optimum concentration of reference solution will be that concentration which gives $a'c_1$ a maximum value.

To establish this optimum concentration for the determination of uranium, a series of uranyl sulfate solutions of known uranium content, ranging from 0 to 60 mg. of uranium per ml., was prepared. One of these solutions, with uranium content c_1, was used as a reference solution to set the spectrophotometer to zero. The relative absorbance, Ar, of a somewhat more concentrated solution of concentration c_2, was then measured. The value of a' was calculated as $Ar/c_2 - c_1$. The process was continued with reference solutions of increasing uranium content, the last containing 60 mg. per ml. Finally, $a'c_1$ values were computed for each pair of liquids of the series. These data are presented in Table I.

Table I. Computation of Optimum Concentration for Uranyl Sulfate Reference Standard

c_1, Mg./Ml.	Δc, Mg./Ml.	Ar	Slit Width, Mm.	a'	$a'c_1$
0	33.4	1.75	0.022	0.052	...
8.28	25.12	1.24	0.080	0.049	0.406
8.35	25.05	1.33	0.084	0.053	0.443
16.55	16.85	0.882	0.110	0.052	0.861
16.70	16.70	0.855	0.120	0.051	0.852
21.77	16.04	0.761	0.165	0.048	1.045
24.75	12.44	0.589	0.22	0.048	1.188
30.9	20.6	0.947	0.53	0.046	1.42
36.1	15.4	0.711	0.68	0.046	1.66
41.2	26.5	0.776	0.88	0.038	1.15
46.4	...	...	1.07	...	...
51.5	51.5	0.743	1.29	0.014	0.72
61.8	41.2	0.522	1.63	0.013	0.80

c_1 = concentration of more dilute standard reference solution.
Δc = difference between concentration of sample solution and c_1.
Δc = $c_2 - c_1$ when c_2 is concentration of more concentrated solution.
Ar = relative absorbance of more concentrated solution measured against less concentrated solution.
a' = $Ar/c_2 - c_1$.

From this table, it is apparent that $a'c_1$ is a maximum when c_1 is of the order of 35 mg. per ml. This, then, is the optimum concentration for the reference solution. However, in order to use a solution of this concentration as a reference solution to set the instrument to zero, it was necessary to use a wide slit, of the order of 0.6 mm. This is not desirable because of the loss of resolution and greater possibility that absorbance bands of other components of the system will overlap the band used for uranium determination. For this reason, a somewhat lower concentration of reference solution, 20 mg. per ml., was selected for use. At this concentration, the width of slit required is only about one fourth that required at 35 mg. per ml., while the value of $a'c$ and, consequently, that of the relative error have been changed by no more than one third.

ESTIMATION OF URANIUM CONCENTRATION IN UNKNOWN SOLUTIONS

The uranium in uranyl sulfate solutions was calculated from test data by means of an equation of a calibration graph, as described by Young and Hiskey (*12*). The equation used is as follows:

$$c_2 = F \times Ar + c_1 \qquad (2)$$

when

c_2 = concentration of uranium in unknown solution, mg. per ml.
Ar = relative absorbance (difference in absorbance of unknown solution and reference standard solution)
c_1 = concentration of uranium in standard reference solution, mg. per ml.
F = factor = average of a number of $\frac{\Delta c}{Ar}$ values

The factor, F, is determined by measuring the difference in absorbance, Ar, of a series of reference solutions and solutions of known and somewhat higher concentration, dividing the difference in concentration, Δc, by the difference in absorbance, and averaging the results (see Table II).

Table II. Calibration Factor for Determination of Uranium by Differential Colorimetry

Uranium, Concentration Range, Mg./Ml.	Slit Width, Mm.	No. of Standards	Average Factor, F	Coefficient of Variation, %
22–38	0.165	6	20.5	2
22–38	0.165	6	20.4	1
22–42	0.165	9	20.5	2
22–50	0.165	13	20.8	2
22–44	0.165	11	20.1	2
22–50	0.165	14	20.2	2
31–52	0.53	4	20.8	3
		Av.	20.5	
Coefficient of variation, %			1	

The concentration of uranium in the standard and unknown solutions may differ by as much as 2 to 4 mg. per ml. without seriously affecting the accuracy of the determination. The term, $F \times Ar$, is assumed to equal $c_2 - c_1$; so long as the error in this assumption does not exceed 0.4 mg. per ml., no larger error will be introduced into the determination of the concentration of the unknown.

CONTROL OF VARIABLES

In precision spectrophotometry, all factors that affect the absorbance to a significant degree must be carefully controlled. Errors due to mismatched cells, dilution effects, and lack of temperature and acidity control often become significant. Accordingly, in this study, all differential absorbance measurements were made with cells that had been carefully matched while filled with highly absorbing solutions. In order to reduce calibration errors to a minimum, all volumetric glassware was calibrated and the same glassware was used in the preparation of reference and test solutions.

Temperature differences may introduce errors by changing the volumes or by altering the absorbance characteristics of solutions. The first effect may lead to concentration errors; the second may produce a change in the slope of the calibration graph and, consequently, in the calibration factor. In case the absorbance characteristics change with temperature, the magnitude of the error due to temperature difference will depend on the temperature sensitivity of the particular colored solution used. Bastian (*4*) demonstrated this relationship for copper perchlorate, potassium dichromate, and potassium permanganate, which differ widely in temperature sensitivity.

Table III. Reliability of Differential Spectrophotometric Method in Determination of Uranium in Uranyl Sulfate Solutions

Uranium, Mg./Ml.		Difference	
Present	Found	Mg.	%
29.1	29.0	−0.1	0.3
30.5[a]	30.4	−0.1	0.3
33.4	33.3	−0.1	0.3
35.0	35.1	+0.1	0.3
35.4[b]	35.2	−0.2	0.6
36.4	36.3	−0.1	0.3
40.4	40.3	−0.1	0.3
46.7	46.8	+0.1	0.2
46.7	46.7	0	0
50.2[c]	50.0	−0.2	0.4
58.4[c]	58.2	−0.2	0.4

[a] Sample contained γ quantities of Cr^{+6}, Fe^{+++}, Ni^{++}, and MnO_4^-.
[b] Sample 0.25N with respect to sulfuric acid.
[c] Uranium concentration of reference standard differed from that of sample by more than 4 mg. per ml.

In the determination of macro quantities of uranium in uranyl sulfate, errors due to temperature difference were avoided by carrying out all operations at essentially a constant temperature. All dilutions of sample and standard reference solutions were made at the same temperature, thus avoiding concentration errors. Relative absorbance measurements were made at essentially the same temperature at which the solutions were processed. It was observed that if no cooling system were used, the temperature of the cell compartment of the spectrophotometer increased 2° per minute. Two different methods were used to maintain the temperature of the sample and reference solution equal and essentially constant. In some tests, a fresh standard reference solution was used with each test sample and relative absorbance was measured rapidly (within 10 to 20 seconds) before the temperature of the cell solutions had increased appreciably. In other tests, a liquid-cooled mounting block and thermospacer attachment was used to maintain the cell solutions at constant temperature. The precision was equally satisfactory with both methods of temperature control. Moderate difference in the temperature at which relative absorbance is measured and at which calibration data are taken, can be tolerated; for a precision of 0.3%, the difference may be as much as 2° C.

As sulfuric acid in appreciable concentrations will alter the absorbance of uranyl sulfate solutions, its concentration must be controlled (*1*). In the study reported herein, however, uranium was determined in uranyl sulfate solutions containing no sulfuric acid other than the small amount produced by hydrolysis. For these solutions, no control of acidity was necessary. Bacon and Milner (*1*), on the other hand, determined uranium spectrophotometrically in strong sulfuric acid solutions. They found that the optimum concentration of acid was 4M and that a 12% difference (increase or decrease) in acid strength produced a negative error of 0.1%. Obviously, in working with solutions containing appreciable amounts of sulfuric acid, the acidity of test solutions and standard reference solutions must be controlled. The difference in acidity which can be tolerated will be determined by the precision desired.

PROCEDURE FOR ANALYSIS OF UNKNOWN

Select a pair of well matched cells for absorbance measurements. Introduce the solution of unknown concentration into one of them and make an approximate determination of its concentration. To do this, adjust the width of the slit until the instrument is set at zero. Then, by reference to a table similar to Table I but prepared for the particular spectrophotometer being used, find the approximate concentration that corresponds to the required slit width. Place a reference solution of somewhat lower concentration than that indicated for the unknown in the second cell, and, with the reference solution in the light path, set the spectrophotometer to zero. Then move the unknown solution into the light path and measure its relative absorbance, Ar. While making the measurement of relative absorbance, control the temperature as described in the section on control of variables. Calculate the concentration of uranium in the unknown by means of the equation, $c_2 = F \times Ar + c_1$, as explained previously.

PRECISION OF METHOD

The test results for a series of samples are presented in Table III, together with the uranium concentration as determined by a gravimetric method. For 11 samples, the average standard deviation is 0.3%. One of these samples contained microgram quantities of impurities, chromate, ferric, nickel, and permanganate ions, and one was $0.25N$ with respect to sulfuric acid. No significant error was caused by the impurities. The presence of acid in relatively high concentrations increased the error.

CONCLUSION

The differential spectrophotometric method provides a rapid and accurate procedure for the determination of relatively high concentrations of uranium. It is especially suitable for solutions which are free from compounds that have strong absorption bands in the wave length region near the 418-mμ peak. Microgram quantities of impurities do not seriously interfere. The presence of sulfuric acid leads to results which are slightly low, unless compensated for.

ACKNOWLEDGMENT

The authors are grateful to H. P. House for his assistance in the preparation of this manuscript.

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Raman Spectrometer Assembled from Available Components

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Raman spectroscopy offers advantages for certain applications, but its advance has been slow, partly because of complicated instrumentation. A Raman spectrophotometer assembled by modifying available components has resolution reproducibility and intensities that make the instrument of practical use.

RAMAN spectroscopy some years ago was the leader in the measurement of vibrational spectra, but it has fallen far behind the infrared in analytical applications. Part of the reason for this lies in problems such as color, fluorescence, solubility, size of sample, and the like, which are inherent in the Raman technique, but generally cause no difficulty in the infrared. Another major reason for the development of infrared rather than Raman spectroscopy has been instrumentation. Infrared has moved far ahead in having commercially available recording spectrometers and spectrophotometers with adequate resolution and precision for adaptation to analytical control as well as structural studies. These instruments have been made compact and rugged enough for satisfactory plant and laboratory operation.

In this approach to the problem, it was decided that any instrument would have to be ready to operate at all times with a minimum of preparation, be rugged enough to be placed in a plant laboratory area, and record fast enough to give a complete spectrum in a reasonable time. The present instrument, made from commercially available parts, is compact and rugged enough to run in a laboratory in the plant area for some time with practically no special precautions. It is ready for use at any time and a spectrum can actually be running within 5 to 10 minutes from notice that it is desired. A complete spectrum can be recorded within a half hour. Its actual performance approaches the achievements of some of the better instruments now in use.

DESCRIPTION OF INSTRUMENT

The basic design of the instrument is similar to that built earlier at the Stamford Laboratories of the American Cyanamid Co. (*4, 5*), but numerous modifications and simplifications have been made.

The light source is a Toronto-type arc (purchased from the Applied Research Laboratories). It is of borosilicate glass and is permanently evacuated. Figure 1 shows the arc mounted in position. The power for the arc is supplied from a direct current source, an available motor generator set of 15 kw., 250 volts, at 60 amperes. This is far in excess of the requirements, as the arc itself has approximately a 100-volt drop at 15 amperes at its optimum operating efficiency (*1*). Any stable direct current source can be used, if it can supply 230 volts at 15 amperes.

Immediately adjacent to the arc in Figure 1 is a double-jacketed glass cylinder. This is a light filter. In the large outside com-

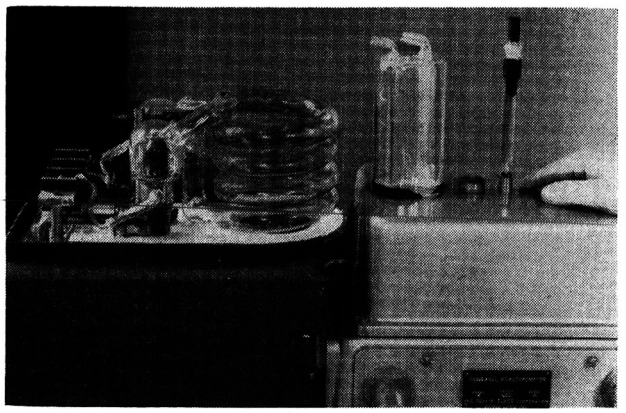

Figure 1. Arc light source, glass double-jacketed light filter cylinder, and sample tube

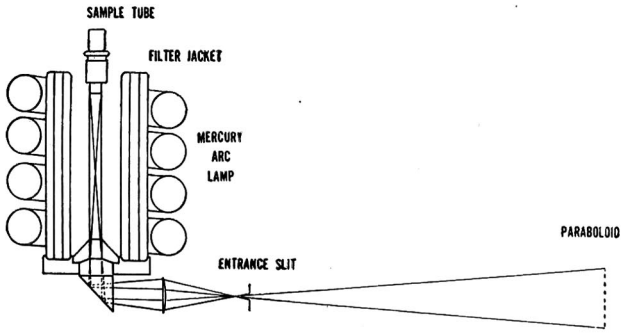

Figure 2. Positioning of light source, light filter, and sample tube with respect to optics of spectrometer

partment saturated sodium nitrite is circulated. This serves a double purpose, inasmuch as it acts as a heat exchanger, keeping as much heat as possible from the sample tube, and as a filter for ultraviolet and violet radiation. The inner and smaller compartment of the filter cylinder may be used for rhodamine, dinitrotoluene, etc.; the choice of the filter solution depends upon the type of sample and the region of the spectrum being surveyed. The 4358 A. mercury line was used to excite the Raman spectra in this installation.

The sample tube is shown in Figure 1 next to the filter jacket. This tube has a concave, back-surface, spherical mirror on the bottom of the stopper which fits into the top of the tube. The radius of curvature is equal to the length of sample tube. The light scattered in both the forward and backward directions is collected in the monochromator; hence the signal is doubled. The sample tube is rigidly positioned so that it is vertical and in the middle of the light source (Figure 2).

The scattered radiation passes through the window at the bottom of the vertical sample tube, impinges on a right-angle prism with the hypotenuse aluminized, and is sent off at right angles in the horizontal direction. A lens then focuses an image of the rear end of the sample tube upon the entrance slit of the monochromator (Figure 2). The light housing which can be seen in Figure 3 was made in the shop and is painted on the inside with a water-base paint of barium sulfate and carboxymethylcellulose, which gives a highly reflective matte finish.

The monochromator is a modified Perkin-Elmer Model 83 universal monochromator. The modifications can best be shown by a schematic drawing of the optical system shown in Figure 4.

The usual aluminized off-axis paraboloid is $M2$. The prism, PR, is of glass and is in the same position as in the standard Perkin-Elmer Model 83. The Littrow mirror has been replaced by an aluminized plane replica grating of 15,000 lines per inch. (Currently a grating of 45,700 lines per inch is being used satisfactorily.) This plane grating is rotated by a modification of the mechanism ordinarily employed for the standard Littrow mirror, but it is oriented so that its dispersion augments that of the prism, and rotation of the grating changes the wave length of the radiation going through the exit slit, $S2$. From the grating the light is further dispersed by another pass through the prism, and it impinges again upon the parabola. The light is then sent to a plane mirror, $M1$, which reflects it through the exit slit. Before going through the exit slit, the light passes through a 13-cycle chopper of special design, the flag being driven by the installation of a special cam upon the standard chopper drive. The position was chosen to permit chopping of the light at its focal point. This resulted in the sharpest rise and decay time of the energy pulses at the photomultiplier detector. The 13-cycle chopping was used because the standard equipment—monochromator and amplifier—was commercially available. The detector, an RCA 1P21 photomultiplier, was mounted behind the exit slit at the focus of the entrance slit image. The photomultiplier voltage was obtained from a regulated power supply (made by Furst Electronics).

Other modifications of the monochromator were: matching the curvature of the entrance slit with the image curvature produced by the prism and grating combination, and installing baffles inside the monochromator, as it was found of primary importance to eliminate as much stray light as possible. The stray light, being the major cause of noise, limits the useful sensitivity of the detector. The monochromator as it was used is shown in Figure 5.

Because the standard 13-cycle chopped light was used, it was feasible to use the standard Perkin-Elmer Model 81 amplifier with only a slight change in the response. In anticipation of high noise levels, 1-, 2-, 5-, and 12-second time constants were made available as compared to the 2-second and less usually found on the Model 81. However, this change was actually superfluous, inasmuch as it has not been necessary to use anything longer than 2 seconds, even though other time constants were tried, as can be seen in Figure 6. Full gain of the amplifier is about 32×.

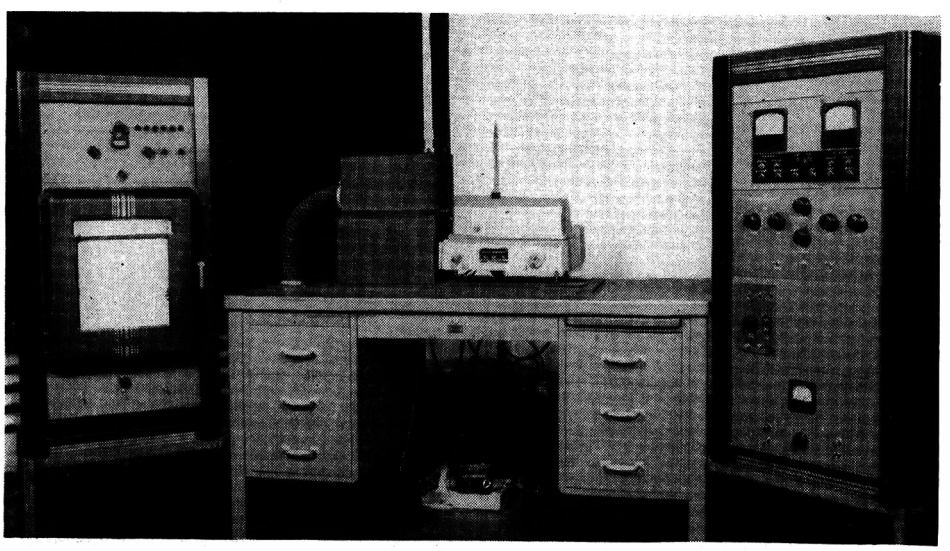

Figure 3. Over-all view of assembled Raman spectrophotometer with controls and recorder

The amplified signal is recorded on a modified extended range Leeds & Northrup Speedomax. The modification consisted of an automatic range changer (built by the Warren Electronics Co.) similar to that described by Salzman (*2*). This range changer makes it possible to record on a standard 10-inch chart roll any signal from less than 1 to 880 mv. The complete assembly is shown to the left in Figure 3.

OPERATION OF INSTRUMENT

Start-Up. The starting or arc-striking process has generally been laborious and time-consuming. In this instrument it was made an automatic time sequence operation, necessitating only the momentary depressing of a contact switch. Basically, the automatic controls consist of a 3-minute interval timer and a direct current relay. The program sequences are started when the button on the main control panel is depressed. This starts a chain of events controlled by interval timer and current relay.

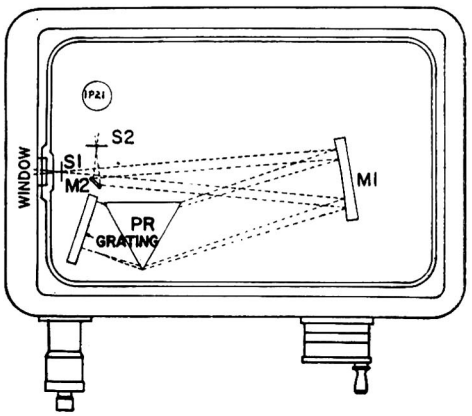

Figure 4. Schematic drawing of optical system of monochromator used in Raman instrument

1. Interval timer starts.
2. Direct current (250 volts) is applied through a suitable dropping resistance for current control of arc lamp.
3. A blower with a capacity of 125 cubic feet a minute is started. (This blower is used for the dual purpose of heating the arc prior to its start and cooling the arc once it has struck.)
4. An electric heater of 4000-watt capacity, which is located in the air duct, is turned on. This heater supplies all the energy that is required to heat the mercury pools and glass helix of the lamp. It will raise the air temperature and the housing from ambient to 100° C. in 10 to 15 seconds and maintain this temperature as long as it is on.
5. The circulation pump for the sodium nitrite filter system is turned on. (This solution is circulated as long as the arc lamp is on, in order to maintain the temperature of the sample tube as low as possible.)
6. At the end of the 3-minute heating cycle, a 7000-volt alternating current is applied across the center and one external electrode of the arc. This potential is maintained for 10 seconds or until the arc strikes (whichever is shorter) and maintains an arc through the lamp.
7. The direct current flow through the arc actuates a current relay which turns off the air heater and high voltage source. It turns on cooling water to fingers in mercury pools of the lamp.
8. After 3 minutes and 15 seconds, the interval timer shuts itself off and resets itself to the starting position.

This program of sequences has unfailingly started the helical mercury arc lamp in this installation.

It is possible to start running a curve within 1 or 2 minutes after the arc comes on, but the amperage drifts somewhat and has to be closely checked throughout the running of the initial spectrum. Within 30 to 60 minutes the current will stabilize and require little or no further attention.

Operating Variables. The most important feature of any analytical technique is its reproducibility. Accordingly, numerous checks of the reproducibility of this instrument have been made. The possible factors causing nonreproducibility have been classified into three main categories—error in sampling, light source, and electronics.

Giving colorless, nonturbid samples, the sampling procedure seems to be almost completely reproducible when all normal precautions are taken and thus should contribute no errors. The deviations accountable to the light source would be of two types. The first would be a gradual error attributed to possible darkening of the tube. This is corrected by running standard curves periodically and applying correction factors. The other possible error is due to variations in the arc current and has to be more or less accepted, unless some monitor system is used. The extent of this error can be estimated, as it has been found that the intensity of a Raman line varies approximately as the current through the light source. The arc system has a minimum amperage deviation of about 0.5 ampere, which means a calculated uncertainty of about $\pm 1\%$ on any line intensity. Actual experiment shows that the average error attributable to the light source is about $\pm 0.5\%$.

The error due to the electronics can be identified as the noise due to the detector, amplifier, or recorder. Actually over 90% of the noise found may be identified as "light noise" in the detector, which is an unrefrigerated 1P21 photomultiplier. The nar-

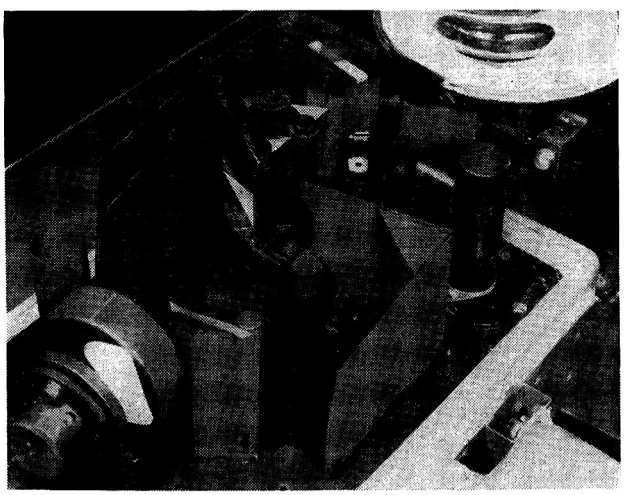

Figure 5. Inside of monochromator

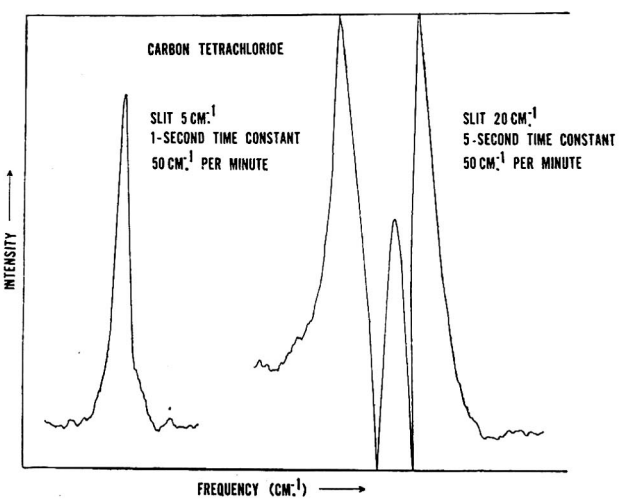

Figure 6. Two Raman spectra of carbon tetrachloride

Left. Slit width 5 cm^{-1}.
Right. Slit width 20 cm^{-1}

row band pass of the alternating current amplifier made it possible to use a photomultiplier of high quality at room temperature. The alternating current component of the dark current is never observed at the amplification levels employed. Some selection of the photomultiplier tube may be desirable.

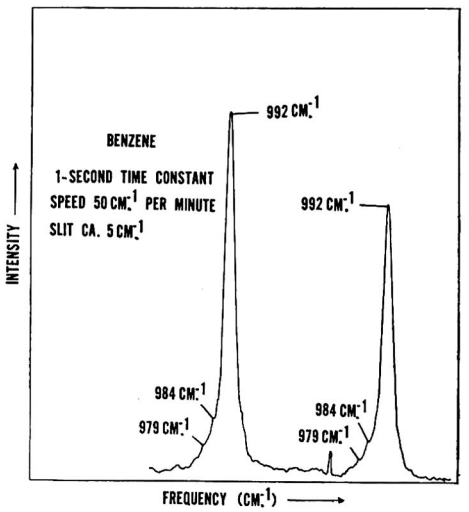

Figure 7. Two Raman spectra curves of benzene

Left. **High amplifier gain**
Right. **Lower amplifier gain**

Actual calculation of this error due to the noise level is rather difficult, inasmuch as the actual ratio of signal to noise is dependent upon the intensity of the Raman line to be studied, and this in turn is dependent upon the slit width used. These factors can best be demonstrated by actual Raman curves. Figure 6 shows the 459-cm.$^{-1}$ line of carbon tetrachloride with a wide (20 cm.$^{-1}$) spectral slit, and the signal-to-noise ratio for this band is well over 500 to 1; on the other hand, next to it is a curve of the same sample of carbon tetrachloride run under identical conditions, except that a 5-cm.$^{-1}$ spectral slit was used. A much lower signal-to-noise ratio of 100 to 1 is obtained, but the band width is much narrower, thus making possible much greater resolution. In referring to noise for signal-to-noise ratios, the maximum noise or randomness found in the background near the peak is used.

Operation of Range Changes. The stronger line of carbon tetrachloride shown in Figure 6 serves to demonstrate the manner in which the range changer works. The peak is on the third scale and has a signal of 45 mv. This is obtained from the range changer going continuously from 0 to 10 mv. on range 1, 10 to 30 mv. on range 2, and 30 to 80 on range 3. There are six ranges, which give a total of 880 mv. Thus it is possible to record signals having ratios of greater than 10,000 to 1 on the same paper at a given amplification setting.

Resolution. The resolution is best shown by a study of the 992-cm.$^{-1}$ line of benzene. Using a wide slit of 20 cm.$^{-1}$, signal-to-noise ratios can be obtained in the neighborhood of 1000 to 1, but no indication of the line due to the carbon-13 isotrope is noted. By going to very narrow slits of about 5 cm.$^{-1}$, the fine structure of the 992-cm.$^{-1}$ line can be resolved partially (Figure 7). The left-hand curve was made at a higher amplifier gain, but in both plots the lines at 979 and 984 cm.$^{-1}$ are resolved.

Reproducibility. If it is recognized that the error in evaluating the true height of a peak is a direct function of signal-to-noise ratio, then the reproducibility has a maximum within about ±1.0% (limited by the light source variations), and probably has no real or absolute minimum value. Taking an average line having a signal-to-noise ratio of about 300 to 1, reproducibility to within about 2 to 3% can be obtained. This is shown in Figure 8, where the 991-, 1004-, and 1030-cm.$^{-1}$ lines of toluene were run three times at approximately 15-minute intervals. The maximum deviation is about 0.25 mv. with a signal of over 8 mv., making an average deviation of about 2%. Weaker lines, of course, have poorer reproducibility, while some stronger lines should reproduce to within ±1.0%.

Figure 9 shows the spectra for a 50% benzene–50% toluene mixture, where the 992-cm.$^{-1}$ line of benzene is easily resolved from the 1004-cm.$^{-1}$ line of toluene. This shows a possible use of Raman spectroscopy in the analysis of mixtures and the detection of small quantities of one in the presence of another. The grating ghost at 991 cm.$^{-1}$ interferes with a direct quantitative measurement. However, this is easily corrected for by knowing the ratio of the intensity of this line to that of the Rayleigh scattered 43,580 line. The actual correction is then obtained by measuring the intensity at 43,580 (or 0-cm.$^{-1}$) and applying the aforementioned ratio. Other analytical applications and

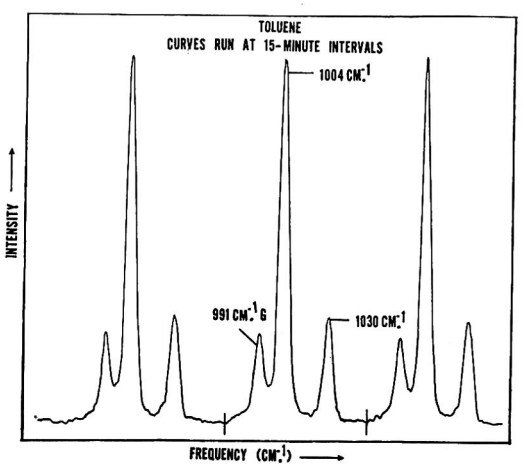

Figure 8. Three Raman spectra curves of toluene run at 15-minute intervals to show reproducibility

991 cm.$^{-1}$ G line is a ghost on this instrument and will be background on other spectra in this region shown in this paper

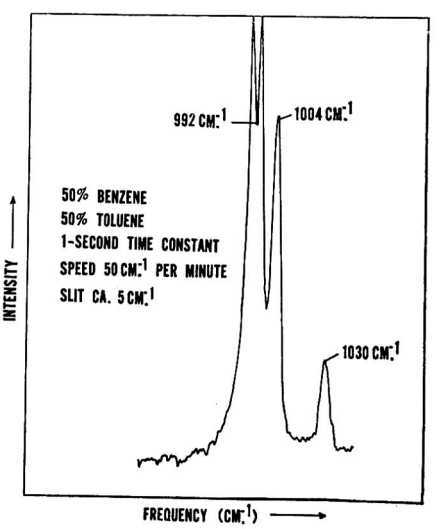

Figure 9. Raman spectra of 50-50 mixture of benzene and toluene

Showing two major lines from these compounds that could be used for analysis

limitations of the Raman technique have been discussed by Stamm (3).

ACKNOWLEDGMENT

The authors wish to acknowledge electronic help received from G. E. Gerhardt and C. W. Warren.

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RECEIVED for review January 20, 1956. Accepted April 14, 1956. Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., 1954, and Instrument Society of America.

Spectrophotometric Titration of Telluric Acid

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As no direct, simple, and precise volumetric methods are available for the determination of telluric acid alone or in the presence of other acids, the possibility of a spectrophotometric titration was investigated. The tellurate ion absorbs at a longer wave length in the ultraviolet than does telluric acid. Therefore, the production of the tellurate ion during an acid-base titration can be followed spectrophotometrically. A plot of absorbance *vs.* milliliters of titrant gives a smooth curve. The linear portions of the curve can be extrapolated to indicate the stoichiometric point of the titration. The spectrophotometric titration serves satisfactorily for the determination of telluric acid alone and in the presence of hydrochloric, sulfuric, nitric, selenic, and acetic acids.

BECAUSE telluric acid is a very weak polyprotic acid, the usual acid-base titration with an internal indicator is not feasible. Rosenheim and Weinheber (4) suggested adding neutral glycerol to the telluric acid solution. The glycerol forms a complex with the telluric acid which behaves as a stronger monoprotic acid. This complex can then be titrated with a standard sodium hydroxide solution using phenolphthalein as the internal indicator. However this method gives results ranging from 96.87 to 103% for a single sample. Another modification also suggested by Rosenheim and Weinheber is the addition of barium chloride to a solution containing the telluric acid sample plus an excess of standard sodium hydroxide. Barium tellurate precipitates and the excess sodium hydroxide is back-titrated with a standard oxalic acid solution. This modification gives results ranging from 98.97 to 100.2%.

Of the oxidation-reduction techniques, the most widely accepted method is the procedure proposed by Gooch and Howland (2). Telluric acid is reduced to tellurous acid by bromide ion in a strongly acid medium. The resulting bromine is distilled into a solution of potassium iodide. The iodine is then titrated with a standard sodium thiosulfate solution, using starch solution as the internal indicator. Although this method is complicated, good results can be obtained.

Scott and Leonard (5) used the absorbance of the tellurate ion in the ultraviolet region for the spectrophotometric determination of telluric acid. Therefore the possibility of spectrophotometrically titrating telluric acid in the presence of other acids with a standard base was investigated.

REAGENTS AND EQUIPMENT

Commercial grade tellurium dioxide was purified by dissolving it in a sodium hydroxide solution, filtering, and reprecipitating the tellurium dioxide by the addition of nitric acid. The purified tellurium dioxide was oxidized with hydrogen peroxide in a dilute solution of sodium hydroxide. The telluric acid was precipitated by the addition of nitric acid, and purified by three recrystallizations from water solutions. Stock solutions were prepared from the purified telluric acid and standardized by the method of Gooch and Howland (2). Stock solutions of the other acids and bases were prepared and standardized. The normality of the ammonium hydroxide solution was checked frequently; no noticeable change was found in its concentration over a month's time. The Beckman Model DU spectrophotometer, equipped with a set of thermospacers, was used with 1-cm. quartz absorption cells for measuring the absorbance of the samples.

RECOMMENDED PROCEDURE

Place the sample of the telluric acid solution in a 250-ml. Erlenmeyer flask and add sufficient water to bring the volume to 50 ml. With an eyedropper transfer a portion of this solution to a quartz cell of 1-cm. light path. With water as a blank, determine the absorbance of the solution. Then return the sample of the acid to the titrating flask by the same method of transfer. Add a small amount of standardized ammonium hydroxide to the flask, stir the contents thoroughly, and transfer a sample to the quartz cell. After each addition of base, use some of the solution to rinse the cell, return it to the flask, again stir the contents, and then transfer a sample to the cell and determine the absorbance. Continue the process for the entire titration. If the volume of titrant added, after the absorbance begins to increase, is greater than 1% of the total volume just before the absorbance increase begins, the absorbance must be multiplied by the dilution factor, $(V + v)/V$.

The corrected absorbances are plotted against milliliters of base added. This type of titration gives a very smooth curve which is easily extrapolated to give the stoichiometric point of the reaction. Klingman Hooker and Banks (3) have shown recently a modification of the cell compartment of a Beckman Model DU spectrophotometer which would speed up the titration as used in this investigation. The cell compartment should be equipped with a set of thermospacers and maintained at the temperature of the solution being titrated.

EXPERIMENTAL AND DISCUSSION

In the ultraviolet region of the spectrum, sodium hydroxide and potassium hydroxide solutions, because of the presence of impurities such as carbonate, were found to have greater absorbances than corresponding solutions of ammonium hydroxide. Therefore ammonium hydroxide was selected as the standard solution in this investigation. The change in the absorption spectrum of telluric acid upon the addition of ammonium hydroxide is shown by curves I and II in Figure 1, while curves II and III show the change with concentration of tellurate ions.

Effect of Wave Length. The effect of wave length upon the ease of detecting the end point was studied. A series of samples, each consisting of 10 ml. of a 0.2*M* telluric acid solution, was

transferred to 250-ml. Erlenmeyer flasks and 40 ml. of water was added. The samples were titrated with standard ammonium hydroxide solution and the absorption of the solution was measured after each addition of the titrant. The change in absorbance during the titration was observed at various wave lengths as shown in Figure 2.

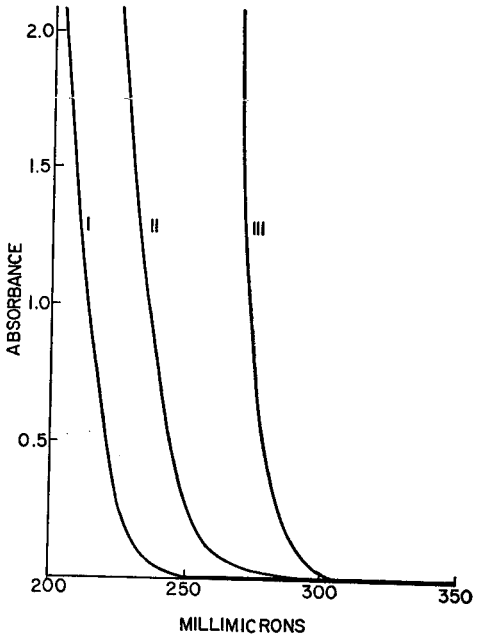

Figure 1. Absorption spectra

I. 20 mg. of telluric acid per 50 ml.
II. 20 mg. of telluric acid with stoichiometric amount of ammonium hydroxide per 50 ml.
III. 500 mg. of telluric acid with stoichiometric amount of ammonium hydroxide per 50 ml.

At the wave lengths studied the telluric acid solutions did not absorb, but upon the addition of the ammonium hydroxide the tellurate ions did absorb. A linear relationship between the absorbance and the milliliters of titrant added was observed. Therefore, as the ammonium hydroxide was added to the telluric acid sample there was a very rapid rise in the absorbance of the solution. Near the stoichiometric point there was a slight rounding off of the curve of absorbance *vs.* milliliters of ammonium hydroxide. However, after the stoichiometric point has been reached, once again the linear relationship of absorbance and milliliters of ammonium hydroxide was established, but this time very little rise in absorbance was observed. The two straight lines could easily be extrapolated to their intersection, thus giving the end point for the titration. At shorter wave lengths than 270 mμ, the molar absorptivity of the tellurate ions is so large that, at the concentrations of telluric acid used in this study, infinite absorbance was reached before the stoichiometric point. Therefore, 270 mμ was the lower wave length limit for this concentration; at longer wave lengths than 290 mμ the molar absorptivity was so small that there was not sufficient difference in the slopes of the lines before and after the stoichiometric point to justify extrapolation.

Optimum Wave Length for Various Concentrations of Telluric Acid. A study of the effect of concentration (Table I) revealed that by changing wave length the titration was made useful for a concentration range between 445 and 11 mg. of telluric acid per 50 ml. of solution. The results obtained for the concentration range showed that the best wave length was approximately 280 mμ for the 445-mg. samples. As the concentration of the acid decreased, lower wave lengths were necessary to assure adequate absorption. To simplify the selection of the proper wave length for an unknown telluric acid sample, the absorbances of a series of telluric acid samples were measured at 240 mμ. Then the samples were titrated at various wave lengths and the optimum wave length was selected for each concentration range (Table II). Thus by merely measuring the absorbance of the telluric acid sample at 240 mμ one can determine from Table II the optimum wave length for the titration.

Concentration of Base. The concentration of base with respect to the concentration of the acid should be in the range of at least 3 to 1, to minimize the dilution error. Excessive dilution of the sample by the addition of the ammonium hydroxide will cause a change in the slope of the curve for absorbance *vs.* milliliters of base. This dilution effect may be corrected by multiplying the observed absorbances by $(V + v)/V$, where V is the volume at the start of the titration and v is the volume added (*1*).

Accuracy and Precision. Analyses of ten separately prepared samples, each containing 96 mg. of telluric acid per 50 ml., gave an average value of 96.22 mg. and a standard deviation of 1.29 mg.

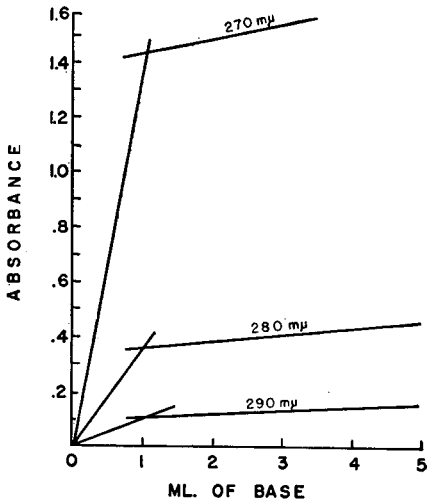

Figure 2. Effect of wave length upon photometric titration curve for 10 ml. of 0.2*M* telluric acid *vs.* 1.937*N* ammonium hydroxide

Table I. Effect of Concentration on Determination of Telluric Acid

Wave Length, Mμ	Acid, Ml./50 Ml. Solution Added	Found	Error, Mg.
280	445.5	445.5	0.00
270	222.5	223.5	+1.0
270	112.5	112.3	−0.2
260	31.23	31.12	−0.11
250	11.25	11.25	0.00

Table II. Selection of Optimum Wave Length for Titration

Absorbance Range for Telluric Acid Sample at 240 Mμ	Optimum Wave Length for Titration, Mμ
Less than 0.075	250
0.075 to 0.190	260
0.190 to 0.340	270
0.340 to 0.500	280

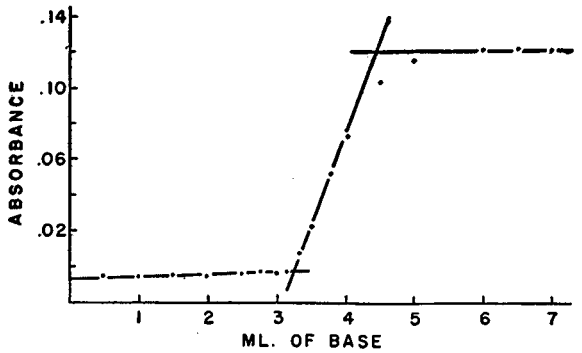

Figure 3. Titration of telluric acid in presence of sulfuric acid

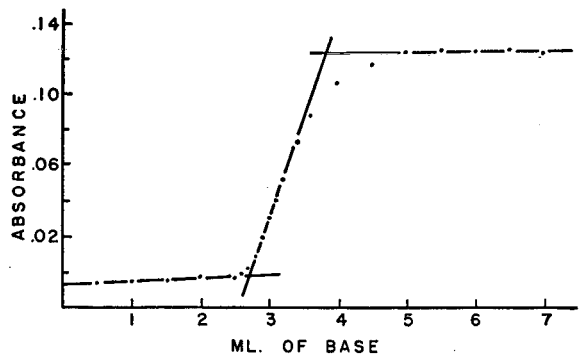

Figure 4. Titration of telluric acid in presence of acetic acid

Titration of Telluric Acid in Presence of Strong Acids. The titration of telluric acid in the presence of other strong or weak acids was studied at a wave length of 270 mμ. Figures 3 and 4 show the general type of curve obtained when telluric acid is titrated in the presence of other acids. There is little change in the absorbance until the stronger acid is titrated. As the telluric acid is titrated, there is a rapid rise in the curve up to the stoichiometric point of the telluric acid titration and then the curve levels off again as more ammonium hydroxide is added. By extrapolating the linear portions of the curve, two breaks are obtained. The first break represents the beginning of the telluric acid titration and the second represents the stoichiometric point. Therefore, the volume of base consumed between these two points corresponds to the volume necessary for the titration of the telluric acid. Table III shows the results of titrations of telluric acid in presence of other acids.

Table III. Titration of Telluric Acid in Presence of Other Acids[a]

Acid Present	Telluric Acid Found, Mg.	Error, Mg.
HCl	107.7	−0.2
HNO_3	107.1	−0.8
H_2SeO_4	107.9	0.0
H_2SO_4	108.6	+0.7
$HC_2H_3O_2$	108.5	+0.6

[a] Each sample contained 107.9 mg. of telluric acid and 5 ml. of a selected acid at approximately 0.3*N*.

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RECEIVED for review September 22, 1955. Accepted April 21, 1956. Abstracted from a thesis submitted by Robert W. Henry in partial fulfillment of the requirements for the degree of master of science, Kansas State College, August 1955.

Identification of *S*-Benzylthiuronium Salts from X-Ray Powder Diffraction Patterns

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The advantages of *S*-benzylthiuronium salts for the analysis of organic acids have been extended by the application of x-ray powder diffraction data as a convenient and reliable means of identification. Several thiuronates, hitherto unreported, have been prepared and their x-ray data given. The use of x-ray analysis is offered as a valuable supplement for melting point determinations in qualitative organic analysis.

THIS paper outlines the results of an application of x-ray diffraction analysis for the identification of some acid derivatives. *S*-Benzylthiuronium chloride is a familiar reagent in the qualitative analysis of carboxylic and sulfonic acids (*10, 11*). The preparation of these derivatives has certain distinct advantages (*6*), which unfortunately are marred by several limitations. In general, the melting points of the derivatives lie within a narrow range (*12*). Moreover, there are marked discrepancies among the published melting points, owing to variations in the rate of heating (*1*) or to the presence of impurities (*7*). Although modifications have been suggested (*3, 5*), the facile accessibility of *S*-benzylthiuronium chloride makes it the more commonly used reagent.

During the course of an investigation relating to the elucidation of certain plant degradation products, this reagent was frequently employed for the identification of thermally sensitive acids in preference to the preparation of other derivatives such as the para-substituted phenylacyl esters, the anilides, or silver salts (*8*). In order to circumvent the disadvantages entailed in its use, the feasibility of adopting x-ray powder diffraction analysis for identification was investigated. The results described prompted this report.

EXPERIMENTAL

The thiuronium derivatives were prepared essentially by the method outlined by Vogel (*11*), except that in some instances it

Table I. Powder Diffraction Data for *S*-Benzylthiuronium Salts of Aliphatic Acids

d, A.	I/I_1
Formic Acid, M.P. 152° C.	
8.08	0.40
6.05	0.40
5.39	0.20
4.69	0.40
4.49	0.40
4.30	1.00
4.02	0.60
3.96	0.60
3.83	0.60
3.65	0.80
3.51	0.50
3.42	0.50
3.23	0.30
3.20	0.30
3.02	0.40
2.91	0.40
2.75	0.40
2.63	0.50
2.39	0.30
2.10	0.30
1.98	0.20
Acetic Acid, M.P. 142° C.	
11.8	0.50
8.28	0.90
5.81	0.80
5.25	0.40
4.91	0.80
4.80	0.40
4.54	0.70
4.49	0.70
3.99	0.50
3.93	0.80
3.76	0.40
3.67	0.40
3.61	1.00
3.54	0.40
3.44	0.50
3.28	0.80
2.86	0.70
2.78	0.50
2.55	0.30
2.28	0.40
2.14	0.40
1.84	0.40
1.72	0.20
Propionic Acid, M.P. 149° C.	
11.7	1.00
7.34	0.70
6.02	0.30
5.25	0.20
4.91	0.50
4.75	1.00
4.61	0.40
4.37	0.60
4.22	0.40
4.15	0.70
4.04	0.80
3.86	0.50
3.79	0.80
3.66	0.90
3.40	0.40
3.12	0.40
2.90	0.50
n-Butyric Acid, M.P. 150° C.	
12.2	1.00
9.08	0.40
8.49	0.50
6.51	0.30
4.67	0.70
4.55	0.80
4.43	0.80
n-Butyric Acid, M.P. 150° C.	
3.92	0.60
3.65	0.60
Isobutyric Acid, M.P. 144° C.	
12.4	1.00
8.49	0.50
4.91	0.80
4.71	0.70
4.59	0.70
3.95	0.60
3.82	0.50
3.66	0.70
3.46	0.40
3.04	0.40
n-Valeric Acid, M.P. 150° C.	
11.3	0.90
9.08	0.30
8.49	0.40
5.67	0.40
4.73	0.60
4.52	0.50
4.45	1.00
3.98	0.50
3.80	0.50
3.66	0.60
3.32	0.40
2.83	0.40
2.61	0.50
2.37	0.30
2.25	0.30
1.91	0.20
Isovaleric Acid, M.P. 153° C.	
10.0	1.00
7.31	0.50
4.90	0.50
4.52	0.60
4.29	0.40
4.04	0.60
3.88	1.00
3.60	0.50
3.36	0.70
3.32	0.70
3.21	0.70
2.93	0.60
2.59	0.70
2.46	0.40
2.09	0.40
1.94	0.30
1.79	0.40
1.74	0.50
1.01	0.50
0.96	0.30
Caproic Acid, M.P. 150° C.	
11.9	0.90
9.09	0.30
8.42	0.30
5.75	0.40
5.57	0.30
4.69	0.80
4.63	1.00
4.53	0.70
3.96	0.50
3.80	0.50
3.79	0.50
3.56	0.40
3.49	0.40
3.15	0.40
Caproic Acid, M.P. 150° C.	
2.83	0.30
2.75	0.20
2.34	0.30
2.26	0.20
Lauric Acid, M.P. 145° C.	
16.5	1.00
7.24	0.30
6.76	0.20
6.16	0.50
5.78	0.30
5.51	0.40
5.13	0.40
4.67	0.90
4.43	0.80
4.15	0.40
4.07	0.40
3.99	0.40
3.92	0.40
3.82	0.40
3.67	0.70
3.61	0.40
3.38	0.40
3.22	0.40
3.11	0.30
2.86	0.50
2.78	0.20
Myristic Acid, M.P. 148° C.	
17.7	0.90
9.09	0.20
7.04	0.10
5.95	0.10
5.42	0.20
5.13	0.40
4.75	0.10
4.63	1.00
4.20	0.50
4.04	0.30
3.82	0.20
3.72	0.30
3.62	0.20
3.49	0.30
3.36	0.30
2.98	0.10
2.39	0.10
Stearic Acid, M.P. 144° C.	
20.1	0.40
13.5	0.50
10.26	0.40
5.75	0.20
5.03	0.40
4.63	0.70
4.22	0.20
4.12	1.00
3.83	0.40
3.70	0.60
3.57	0.50
3.11	0.20
2.47	0.20
2.20	0.20
Behenic Acid, M.P. 135° C.	
11.7	0.50
5.78	0.20
4.91	0.30
4.63	1.00
4.22	0.40
3.83	0.20
3.62	0.30
1.17	0.40
1.11	0.20

was preferable to dissolve the acids in 15 to 20 ml. of ethyl alcohol before neutralizing with 1*N* sodium hydroxide. The crystalline products were recrystallized from ethyl alcohol, dioxane, ethyl acetate, or methyl Cellosolve. Mixtures in solution were prepared by dissolving weighed amounts of the acids in the appropriate solvent, neutralizing with alkali, and adding to a chilled aqueous solution containing an equivalent amount of *S*-benzylthiuronium chloride. Elemental analysis of the thiuronates was made in the laboratories of Robert Dietrich, Zurich, Switzerland.

The melting points were determined by means of a calibrated Fisher-Johns block. The rate of heating was controlled so that the temperature increase was kept at 1° C. per minute near the melting point.

The x-ray powder diffraction data were obtained with a Norelco x-ray diffractometer. Iron-filtered cobalt radiation

Table II. Powder Diffraction Data for Polyfunctional *S*-Benzylthiuronium Salts

d, A.	I/I_1
Oxalic Acid, M.P. 200° C.	
11.2	0.70
7.04	0.20
5.95	0.60
5.57	0.40
5.05	0.30
4.75	0.30
4.55	0.80
4.36	0.90
3.96	0.80
3.71	0.80
3.62	0.30
3.53	0.40
3.36	0.50
3.28	1.00
3.10	0.40
2.99	0.50
2.84	0.50
2.74	0.30
2.57	0.30
2.53	0.40
2.50	0.40
2.34	0.30
2.29	0.30
2.24	0.30
2.19	0.30
2.07	0.20
1.85	0.20
1.81	0.30
1.79	0.30
1.71	0.20
Adipic Acid, M.P. 154° C.	
12.8	0.50
10.3	0.60
7.04	0.50
5.72	0.40
5.15	0.40
4.73	1.00
4.63	0.90
4.49	0.60
4.34	0.60
4.23	0.60
4.15	0.80
3.92	0.70
3.83	0.60
3.66	0.70
3.44	0.40
3.23	0.40
3.12	0.50
2.91	0.50
2.76	0.40
2.56	0.50
2.41	0.50
2.36	0.50
2.25	0.40
2.06	0.40
1.84	0.20
Glutaric Acid, M.P. 162° C.	
13.2	0.70
8.02	0.30
6.05	0.50
5.36	0.30
4.82	0.60
4.59	0.60
4.36	1.00
4.27	0.90
3.98	0.40
3.65	0.30
3.40	0.30
3.26	0.40
3.12	0.40
3.05	0.40
2.76	0.50
2.57	0.40
2.33	0.20
Benzoic Acid, M.P. 178° C.	
10.58	0.70
5.81	0.40
5.31	0.40
4.96	0.30
4.89	0.30
4.73	0.80
4.61	0.80
4.32	1.00
4.12	0.50
3.89	0.50
3.72	0.50
3.56	0.40
3.48	0.20
3.41	0.40
3.17	0.60
3.02	0.20
2.89	0.30
Benzoic Acid, M.P. 178° C.	
2.83	0.40
2.71	0.30
2.54	0.20
2.39	0.30
2.29	0.20
Salicylic Acid, M.P. 149° C.	
10.9	0.50
10.1	0.50
6.16	0.30
5.98	0.30
5.39	0.40
5.10	0.30
5.03	0.20
4.96	0.90
4.71	0.40
4.63	0.40
4.15	1.00
3.98	0.40
3.79	0.40
3.20	0.20
3.00	0.20
2.97	0.20
m-Hydroxybenzoic Acid, M.P. 162° C.	
11.2	0.60
8.85	0.30
5.10	0.80
4.96	0.30
4.41	1.00
4.29	1.00
4.10	0.50
3.90	0.40
3.76	0.50
3.68	0.40
3.18	0.30
2.73	0.30
2.67	0.40
2.31	0.20
p-Hydroxybenzoic Acid, M.P. 144° C.	
10.5	0.60
6.85	0.30
6.02	0.30
5.36	0.40
5.23	0.40
4.77	0.60
4.63	0.70
4.25	1.00
3.93	0.60
3.75	0.40
3.66	0.40
3.14	0.50
2.81	0.40
2.66	0.30
2.36	0.40
2.30	0.40
2.21	0.50
1.99	0.30
1.64	0.20
α-Alanine DNP[a], M.P. 186° C. Calcd. for $C_{17}H_{18}O_8N_8S$. N, 16.66. Found. N, 16.44	
10.7	0.40
9.51	0.30
8.85	0.60
6.68	0.80
6.47	0.50
6.23	0.50
5.81	0.30
5.26	0.40
4.79	0.40
4.32	0.40
4.04	0.50
3.89	0.40
3.71	0.60
3.52	1.00
3.21	0.40
3.16	0.40
2.83	0.40
2.73	0.40
2.65	0.30
2.55	0.40
1.93	0.30
1.84	0.30
β-Alanine DNP[a], M.P. 147° C. Calcd. for $C_{17}H_{18}O_8N_8S$. N, 16.66 Found. N., 16.32	
13.3	0.40
9.08	0.20
β-Alanine DNP[a], M.P. 147° C. Calcd. for $C_{17}H_{18}O_8N_8S$. N, 16.66 Found. N, 16.32	
7.29	0.50
6.63	0.40
6.02	0.80
5.20	0.50
4.75	0.30
4.52	0.50
4.28	0.90
4.20	0.90
4.01	1.00
3.76	0.70
3.54	0.50
3.49	0.60
3.28	0.20
3.08	0.70
3.03	0.70
2.73	0.20
Benzenesulfonic Acid M.P. 147° C.	
8.70	0.50
8.42	0.60
5.95	0.50
5.72	0.30
5.18	0.50
4.73	1.00
4.28	0.60
4.22	0.80
3.80	0.60
3.62	0.70
3.51	0.50
3.49	0.50
3.26	0.20
3.06	0.40
2.84	0.50
2.83	0.40
2.51	0.40
2.11	0.20
1.78	0.20
Benzenearsonic Acid, M.P. 150° C. Calcd. for $C_{22}H_{27}O_8N_6S_2As$. N, 10.47. Found. 10.13	
17.9	1.00
9.08	0.20
6.68	0.70
6.42	0.50
6.09	0.40
5.36	0.70
5.15	0.70
4.53	0.80
4.19	0.30
3.62	0.50
3.50	0.30
3.33	0.60
3.12	0.50
2.90	0.60
2.51	0.60
1.79	0.20
Benzenephosphonic Acid, M.P. 190° C. Calcd. for $C_{22}H_{27}O_8N_6S_2P$. N, 11.42. Found. 11.25	
17.99	1.00
8.93	0.60
6.51	0.40
4.69	0.50
4.51	0.50
4.43	0.40
4.36	0.70
4.13	0.20
3.91	0.30
3.76	0.70
3.44	0.40
3.21	0.20
2.61	0.30
2.45	0.30
2,4-Dinitrophenol, M.P. 101° C. Calcd. for $C_{14}H_{14}O_5N_4S$. N, 15.95. Found. 15.78	
17.4	0.80
8.70	0.30
5.75	1.00
4.95	0.40
4.55	0.20
4.39	0.60
4.27	0.20
3.40	0.60
3.36	0.60
3.29	0.70
3.20	0.60
3.09	0.20

[a] 2,4-Dinitrophenyl derivative.

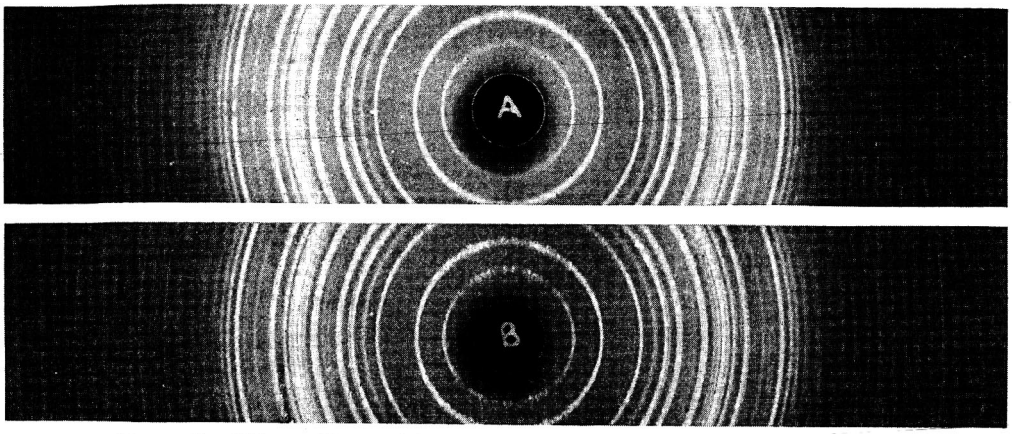

Figure 1. Effect of impurities on x-ray powder diffraction patterns of *S*-benzylthiuronium salts

A. Acetic acid *S*-benzylthiuronium salt recrystallized. % N found, 13.00. % N calcd., 12.96. M.p., 142° C.
B. Acetic acid *S*-benzylthiuronium salt. % N found, 11.66. M.p., 140–141° C.

($K\alpha$ = 1.7889 A.) at 45 kv. and 10 ma. was found to give better dispersion than the more commonly used copper radiation. Diffractometer patterns were used as preliminary guides and the line intensities were estimated visually from powder photographs. Powder photograph intensities were, in general, more reliable and reproducible than diffractometer intensities, presumably because of preferred orientation of the crystals in the diffractometer sample. Exposures were made with Philips cameras 114.5 mm. in diameter, the exposure times varying from 7 to 24 hours. The powder samples were mounted in fine glass tubes of 0.2-mm. bore.

RESULTS AND DISCUSSION

The variety of organic acids listed in Tables I to III were selected to emphasize the wide applicability of the thiuronium derivatives. The data illustrate the distinct value of powder diffraction patterns as reliable criteria for identification. This is particularly evident in the homologous series of the aliphatic acids (Table I) and in the behavior of the structural isomers—for example, valeric and isovaleric acids. Trace impurities have negligible effect on the powder patterns (Figure 1). This confirms previous published results (*8*).

In contrast with other studies (*9*), x-ray analysis is not a reliable method for the qualitative analysis of certain acid mixtures. The data shown by the two-component systems myristic acid–lauric acid and valeric acid–caproic acid (Table III) suggest that the interplanar spacings of these mixtures (prepared in solution) are altered noticeably from those exhibited by their pure components. In fact, they possess properties indicative of complex formation or a solid solution. Mixtures prepared mechanically (Table III)—that is, by grinding the pure components together—showed diffraction lines that can be readily attributed to the presence of either or both components.

The derivatives listed in Table II include several salts hitherto not published. It is evident that the use of *S*-benzylthiuronium derivatives is not restricted to the simple carboxylic or sulfonic acids.

Inasmuch as certain organic derivatives exhibit polymorphism (*4*), and in view of the dimorphic property of the *S*-benzylthiuronium chloride (*2, 11*), efforts were made to prepare crystalline isomers of the thiuronates. Despite repeated recrystallizations from several solvents under various conditions, stable polymorphic forms were not isolated.

Table III. X-Ray Diffraction Data for Mixtures

Thiuronium Derivatives of Myristic Acid–Lauric Acid Mixtures

d, A.	I/I_1
90% Lauric Acid[a], M.P. 142° C.	
16.5	1.00
5.18	0.10
4.67	0.90
4.53	0.90
4.05	0.40
3.87	0.20
3.70	0.60
3.43	0.30
75% Lauric Acid[a], M.P. 144° C.	
16.8	1.00
9.33	0.20
8.63	0.30
6.76	0.40
6.16	0.30
4.98	0.40
4.65	0.90
4.51	0.70
4.23	0.30
3.87	0.60
3.71	0.50
3.57	0.60
3.38	0.30
3.07	0.50
2.76	0.30
2.34	0.20
2.16	0.20
75% Lauric Acid[b], M.P. 137–139° C.	
16.5	1.00
7.09	0.20
6.76	0.20
6.16	0.40
5.51	0.30
5.13	0.30
4.65	0.80
4.49	0.30
4.41	0.80
4.00	0.20
3.82	0.30
3.67	0.70
3.62	0.40
3.38	0.30
3.21	0.30
3.13	0.20
50% Lauric Acid[a], M.P. 145.5° C.	
17.4	0.90
4.61	1.00
4.20	0.20
3.35	0.10
10% Lauric Acid[a], M.P. 146.5° C.	
17.7	1.00
6.31	0.10
5.13	0.30
4.61	0.90
4.20	0.40
4.02	0.30
3.72	0.20
3.60	0.20
3.49	0.40
3.35	0.30
2.33	0.20
Thiuronium Derivative of *n*-Valeric Acid–Caproic Acid Mixtures. 66% *n*-Valeric Acid[a], M.P. 155° C.	
11.7	0.90
8.49	0.20
5.67	0.30
5.56	0.20
4.69	0.50
4.54	1.00
3.96	0.40
3.85	0.40
3.74	0.40
3.50	0.30
3.43	0.40
2.82	0.10
50% *n*-Valeric Acid[a], M.P. 156° C.	
11.7	0.80
9.12	0.30
8.49	0.40
5.75	0.40
5.57	0.30
4.73	0.60
4.54	1.00
3.96	0.60
3.86	0.50
3.75	0.50
3.52	0.50
3.41	0.60
3.15	0.30
2.96	0.10
2.83	0.20
2.65	0.20
2.35	0.30
2.16	0.20

[a] % by weight.
[b] Mechanical mixture, % by weight.

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RECEIVED for review January 13, 1956. Accepted March 27, 1956. Contribution No. 301, Chemistry Division, Science Service.

Laboratory for Remote Analysis of Highly Radioactive Samples

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The function of the Idaho Chemical Processing Plant is to recover enriched uranium from expended fuel elements from various types of reactors. The Remote Analytical Facility provides facilities for chemical research on extremely radioactive materials and for around-the-clock analytical service for operation of the processing plant. This paper describes the general building layout and some typical remote analytical apparatus.

THE Remote Analytical Facility, part of an expansion program at the Idaho Chemical Processing Plant, was completed in May 1955. It provides new facilities both for technical development of new processes dealing with expended fuel elements and for analytical process control.

The building, of reinforced concrete, was constructed adjacent to the main plant. Its area of 88 by 83 feet is divided into three parallel areas shown in Figure 1. The first contains analytical laboratories, the second decontamination apparatus, and the third a multicurie cell. All three areas open into a main corridor which is parallel to and connected with the hot sampling corridor of the main plant.

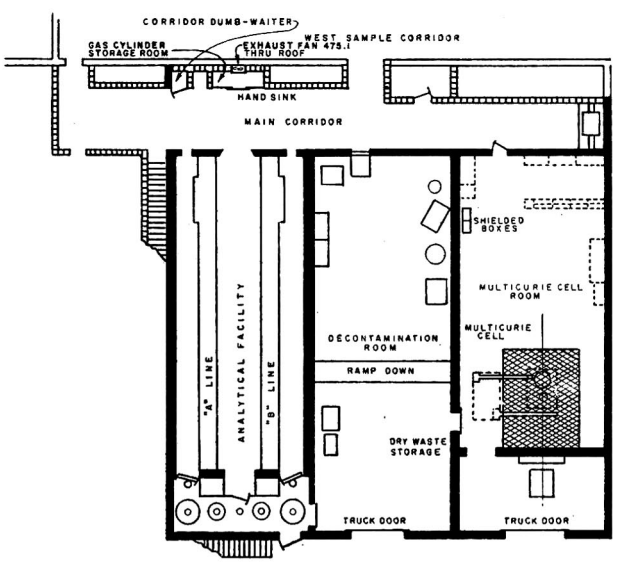

Figure 1. First floor plan of Remote Analytical Facility

This paper is concerned mainly with the analytical laboratories. The decontamination area is briefly discussed later. The multicurie facility is designed to handle 5×10^4 curies of 1.6-m.e.v. gamma or 1.5×10^3 curies of 2.55-m.e.v. gamma activity, as well as irradiated plutonium. This area will be used for investigation of new processes involving uranium recovery from spent fuel elements and the utilization of specific fission products from both uncooled fuel elements and from various plant process streams. The multicurie cell is not described in this paper, for the functions of this facility differ greatly from those of the analytical laboratories. A paper devoted exclusively to the multicurie facility is now in preparation.

The analytical area contains both a remote laboratory and a conventional laboratory. The remote laboratory consists of two parallel lines of Berkeley-type boxes. A heavy shielding wall extends the length of each line, protecting the analysts who operate the equipment that is within the boxes. The equipment is operated by three means: hand-operated manipulators extending through the shielding, pneumatic controls, and electronic controls. Figure 2 is a photograph of these lines, and Figure 3 is a cross section through the lines.

The conventional laboratory is located on the second floor of the Remote Analytical Facility, directly above the remote lines. It serves two main purposes: analysis of samples that do not demand shielding protection and execution of preliminary processing steps for analyses carried out in the remote lines. In a few cases, final processing steps are performed in the conventional laboratory after the constituent to be analyzed is separated from the fission products in the remote line. A photograph of the conventional laboratory is shown in Figure 4.

REMOTE LABORATORY

General Features. The basic unit of the remote laboratory is the analytical box, one of which is shown in Figure 5. These boxes, constructed of Formica-covered plywood, are approximately 3 feet on edge. They are remotely replaceable in the line.

The top half of each box face, slanted back 15°, has a rectangular glass-covered cutout. A high-density window in the shielding wall fits this opening, providing the analyst with a good view of the equipment within the box. The bottom half has two smaller cutouts into which the hand-operated manipulators fit. There is still a smaller opening, between the manipulator openings, which provides access to the box for control rods and reagent lines. The floor of the box is covered with a metal tray in which there is an opening with a hinged door. Samples, glassware, and other material are passed into and out of this opening from a dolly traveling on rails below. A drain, for contaminated liquid waste, is located at the rear of the box.

Two sodium vapor lamps are mounted on top of the box above small windows. One provides sufficient illumination, the other is standby. Inside each box is a panel providing 110-volt regulated, 110-volt unregulated, and 220-volt power. Seven AN-type connectors of various sizes are fastened to a second panel. These provide a total of 52 wires for instrumentation. Figure 6 is a photograph of the inside of a box showing these panels.

Utilities piped into the box are water, vacuum, compressed air, propane, nitrogen, hydrogen sulfide, and a water drain. All utility piping and electrical and instrument conduits rise vertically from the box, make a right-angle bend, and terminate at quick disconnects in line with the box front.

Each box is equipped with pneumatic windshield wipers for cleaning the viewing window.

Air flows through the box at the rate of two changes per minute. It enters through a 1-inch-thick fiberglass filter at the rear of the box and is pulled out through a 3-inch duct at the top.

Sixteen such boxes are in each of the two remote lines. Figure 7 also shows that these lines extend from the main corridor that connects to the main plant to an 8-foot-wide passageway at the opposite end of the new facility. Vertical H-beam columns are spaced along the lines behind the shielding. Heavy angle iron guides fastened to these H columns support the boxes in position.

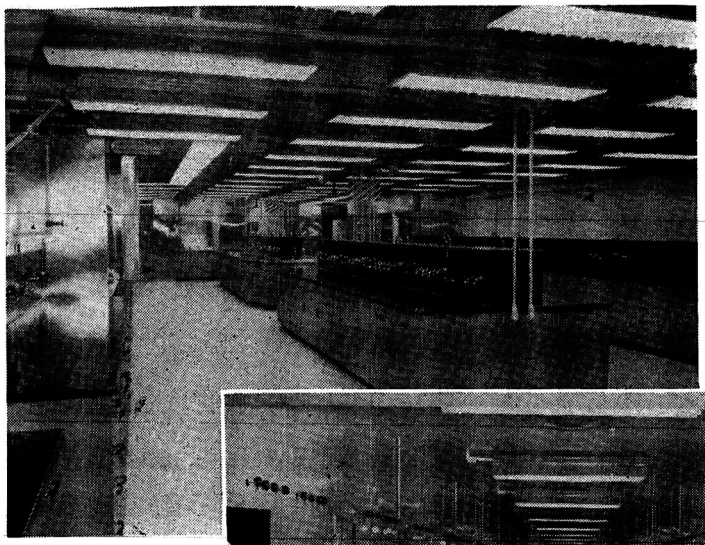

Figure 4. Second floor conventional laboratory

Figure 2. Operating aisle of remote laboratory

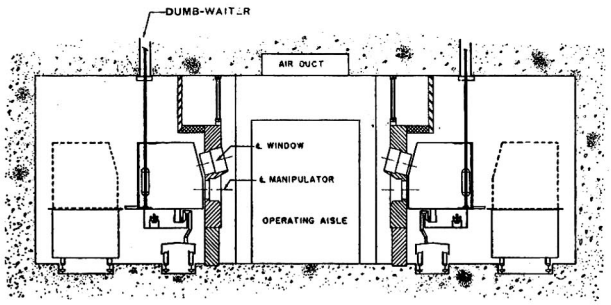

Figure 3. Section through remote laboratory

Boxes are replaceable and are transferred by remotely operated dollies that travel on 2-foot-gage railways behind each line. Figure 8 shows a box being placed in a line. The dolly is positioned by controls on the shielding face, two rods are inserted through holes in the shielding and screwed into receptacles on the box, and the box is pulled into position along the angle guides. The box dolly rails run to the 8-foot passageway, change direction by means of turntables, and terminate in the decontamination area. Here boxes are cleaned of activity and reassembled for future use.

Two additional railways, one for each line, are located beneath the box positions, as shown in Figure 9, and extend the length of the remote lines. Sample dollies, as shown in Figure 10, travel these rails. These dollies provide the means of transfer between boxes. Samples, reagents, glassware, and solid waste are transferred in special holders the size of 1-quart ice cream cartons. Each dolly can carry six cartons. A stainless steel trough extends the length of each line. Contamination resulting from a spill can be washed to drainage with nitric acid and water ejected from a perforated pipe.

Six Lucite rods extend through the shielding at each of the box locations. A beam of light from the sample dolly lights these to designate the ice cream carton in line with the rectangular opening in the bottom of the box.

Both the box and sample dollies are electrically controlled from combined control stations. These stations are located on the shielding wall between each box, as can be seen in Figure 11.

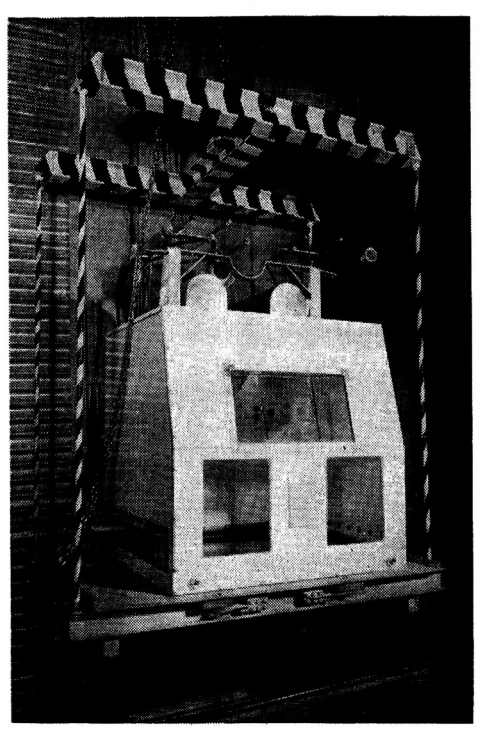

Figure 5. Analytical box and its cradle

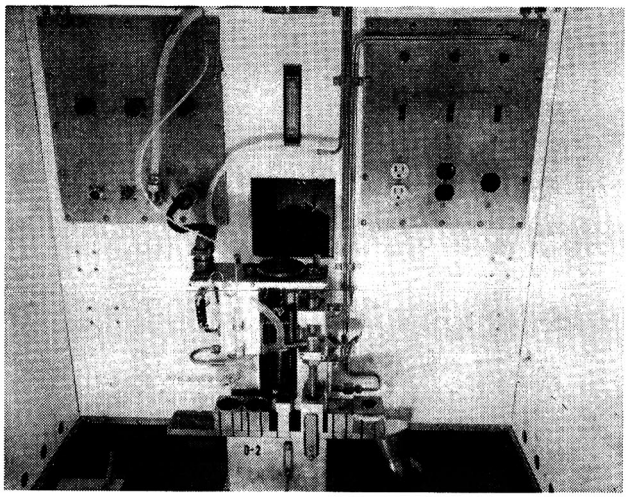

Figure 6. Box electrical and instrument panels and remote pipetter

The sample dolly circuit has an automatic interlock which inactivates all control stations except the one in use.

A small, hand-operated conveyor is also installed which serves only the first four boxes. These boxes are used more than are the remaining 12, especially for incoming samples.

The line shielding is designed to reduce the radiation from a 35-roentgen-per-hour source of 2.18-m.e.v. gamma intensity 12 inches from the inner surface to a maximum of 1 milliroentgen per hour at the outer surface. The shielding, in general, consists of a 9-inch-thick wall of high carbon cast iron (meehanite) with 2 feet of concrete above and to the rear of it. Cross sections of this shielding are shown in Figures 3 and 9. Boxes are separated by 2 inches of lead. The viewing window set in the shielding is made of high-density glass.

Pairs of castle manipulators are set in the shielding. These can be seen in Figure 11. The body of these manipulators is made of lead to provide shielding protection equal to the permanent wall. The manipulator tong can be rotated 45° about the vertical and horizontal center line of the manipulator. The tong can be unscrewed from the tong end by means of a special holder mounted on the rear wall of the box. This allows for remote installation or removal of a box even though the manipulator openings are sealed by flexible, air-tight tygon tubes and bellows. Figure 12 shows how the tube section encloses the tong and the connection of the bellows to the box front.

A removable lead plug serves to seal the small opening between the manipulators. This opening, as previously mentioned, provides access for mechanical control rods and reagent lines to the box. The lead plug usually is constructed with passageways for the tubes and rods.

Six feet above floor level the shielding narrows to 4 inches, and is recessed 21 inches to form a utility chase. Sliding doors, shown in Figure 11, provide an easy means of entry to the chase. When a box is pulled into position, its piping and conduits fit into holes in the 4-inch shielding. Hanson disconnects serve to engage the

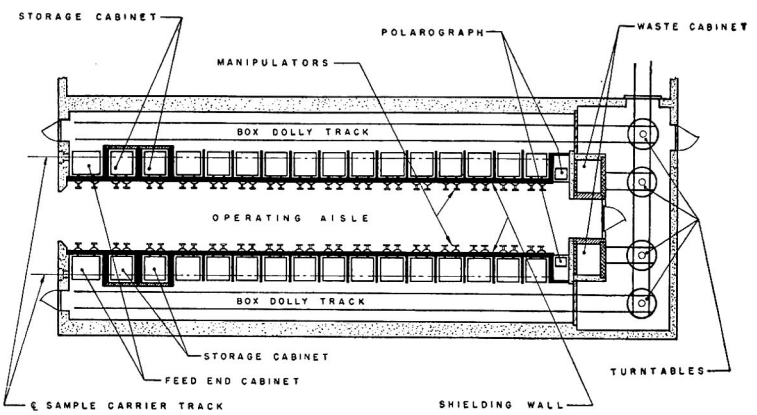

Figure 7. Plan view of remote laboratory

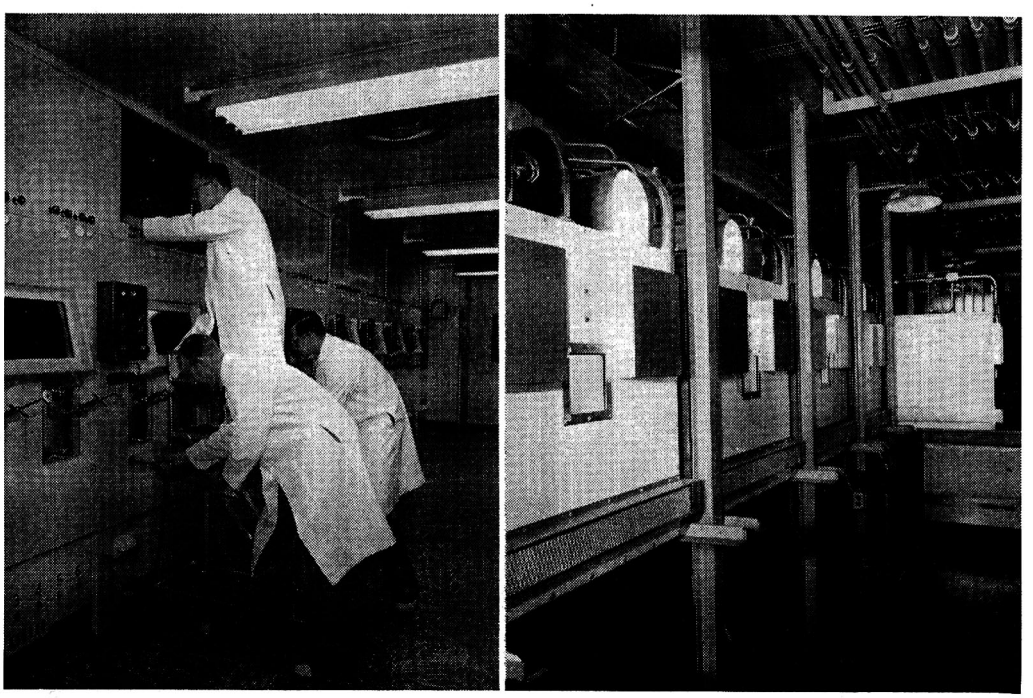

Figure 8. Installation of analytical box

Front and rear views

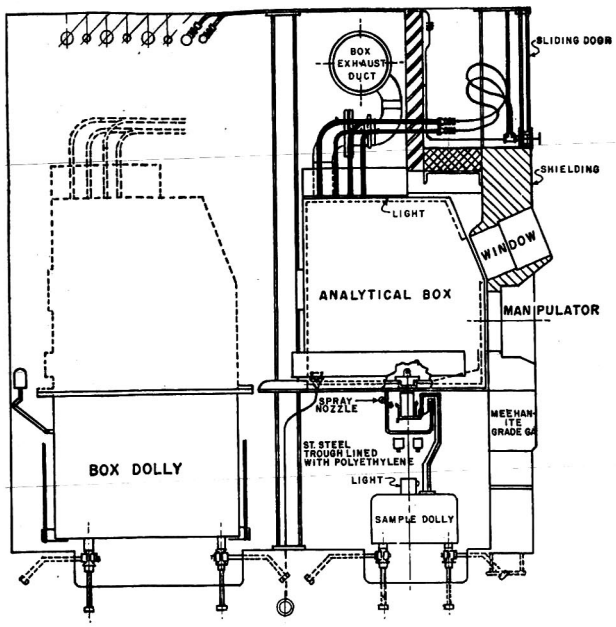

Figure 9. Section through remote line

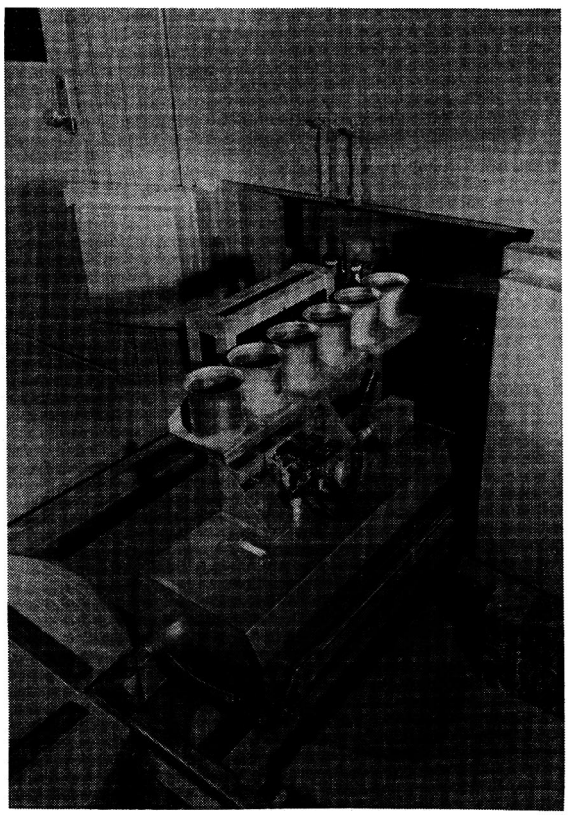

Figure 10. Sample dolly

utility piping; electrical, instrument, and the sodium vapor light connections are made with AN-type connectors. The valves for rate control of the utilities are located just beneath the sliding doors.

The controls and recording units for the analytical apparatus within the boxes are mounted on the shielding face. As can be seen in Figure 11, the control units are uniform in appearance.

The air flow in the remote laboratory is such that contaminated air cannot escape into the operating aisle between the two lines. Air enters the operating aisle from ceiling vents and leaves at floor level through grills in the line shielding. It then passes to the rear of the boxes, and, as previously described, is pulled through and out the top of the boxes. It is finally discharged to the atmosphere through a stack. The air flow through the operating aisle is at the rate of about ten changes per hour.

Box Functions. Each box fulfills a specialized function. The one nearest the main corridor is called the feed-end box. It serves only as a transfer area. As shown in Figure 13, plant samples in lead pigs are brought into the main corridor on manually operated trucks. A sectioned lead door in the corridor wall provides access to the line. The operator aligns the truck rails with rails beneath the feed-end box and pushes the lead pig in. The samples, in 5-ml., cone-shaped, glass bottles, are removed with a manipulator. They are then transferred, usually via the hand-operated conveyor, to one of the next two boxes for storage.

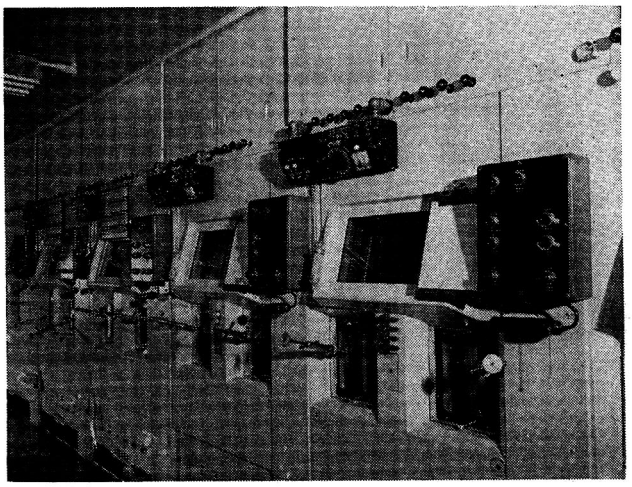

Figure 11. Remote line

A dumbwaiter connects the feed-end box to the conventional laboratory above. This dumbwaiter, with a capacity of four 1-quart ice cream cartons, provides the means by which reagents, glassware, and other materials are transferred into the remote lines and by which treated samples free of fission products are passed upstairs for further processing.

Each of the two storage boxes contains a heavy, cast iron rack which is seen in Figure 14. Each rack provides storage, as well

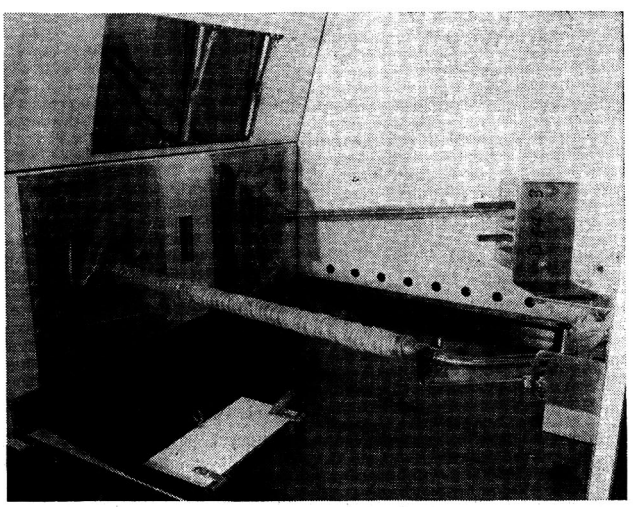

Figure 12. Manipulator tongs inside box

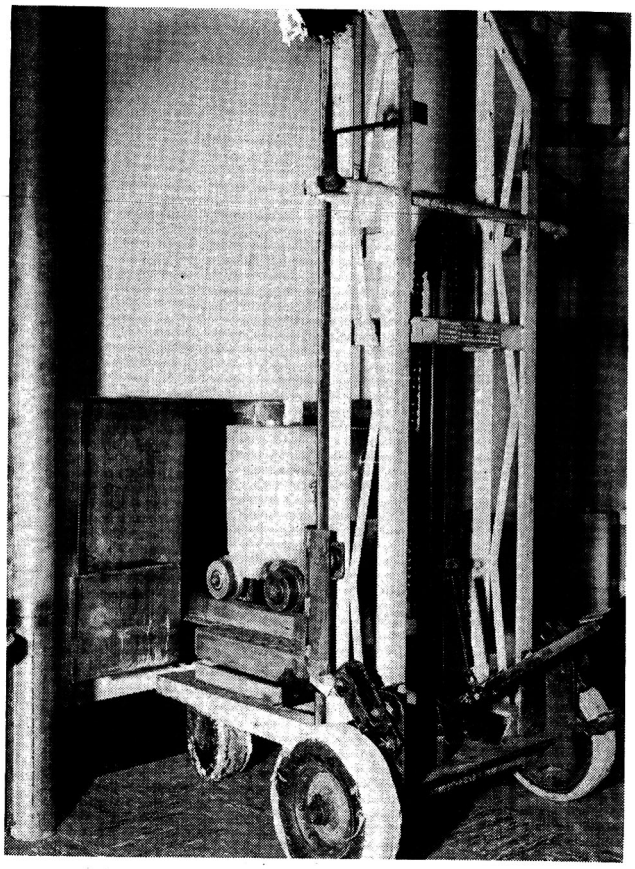

Figure 13. Sample carrier and truck

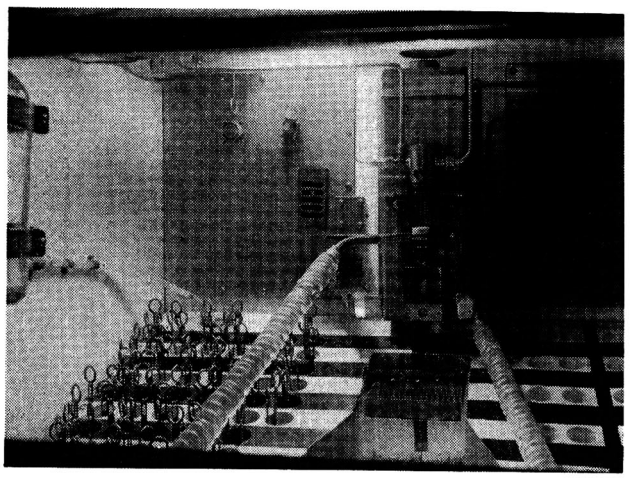

Figure 14. Interior of sample storage box

as additional shielding, for 250 samples of about 35-roentgen-per-hour radiation each. Figure 15 shows the lead shielding behind these two boxes, which is necessary to protect personnel in the adjacent laboratory area or a worker who may be doing emergency work behind the line. Chain hoists are provided to remove this lead shielding in case the box must be replaced.

Each storage box is equipped with a sample residue disposal unit. This device enables the analyst to discard the unused portion of the sample to suitable drainage. Figure 14 shows a portion of this unit. Two sharpened hollow needles pierce the neoprene gasket on the sample bottle when it is pneumatically raised. Rinse water is ejected through one needle, the other pulls, by vacuum, the solution into a liquid trap. The liquid trap is equipped with electronic controls which empty it to drainage when it fills to a certain level.

The remaining 13 boxes in each line are standard units which are equipped with specialized analytical apparatus. Each box serves for a different analysis.

Examples of Analytical Equipment within Boxes. REMOTE PIPETTER. A sample pipetter is installed in the first standard box (Figure 6). This pipetter is a positive displacement, servo-motor type patterned after the models originally installed in the Chemical Processing Plant by the Oak Ridge National Laboratory.

Various receptacles, such as sample bottles, test tubes, and beakers, are positioned beneath the delivery needle by means of a slide. The slide is moved by a manually operated rod passing through a lead plug in the access opening between the manipulators. The pipetter is moved up and down by an air cylinder, the control of which is mounted on the shielding face.

The unit, made of stainless steel, basically consists of a close tolerance barrel with a screw-driven plunger. When the plunger is in the up position, a hole in it lines up with one in the barrel. Samples are drawn into the barrel by lowering the whole unit until the needle tip pierces the neoprene gasket of the sample bottle and then applying suction with a hand-operated syringe mounted on the shielding face. After all of the desired sample aliquots are delivered, the plunger is raised until the holes again align. Acid, water, and acetone are pulled by vacuum through the unit to ready it for the next sample. These liquids pass into a liquid trap similar to that already described for the sample residue disposal units.

The movement of the plunger is controlled by a servo-motor actuated from a Brown electronic continuous balance unit. Two 10-turn Helipots are built in, one connected to the plunger servo-motor, the other to an indexing dial which serves as the control. The unit is so constructed that one complete turn of the control dial results in the delivery of 0.1 ml. of sample.

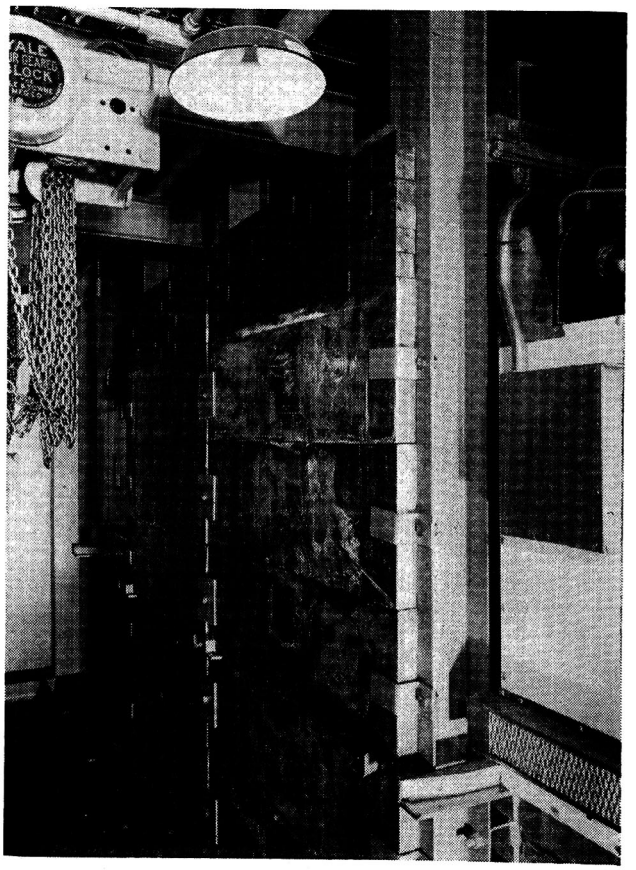

Figure 15. Shielding behind sample storage box

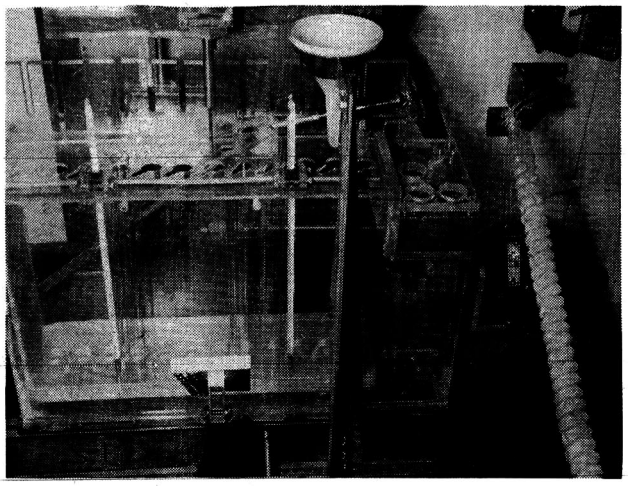

Figure 16. Specific gravity apparatus

This pipetter will deliver any desired volume up to 0.8 ml. Precision is of the order of 0.2%, expressed as the standard error of a single delivery.

Specific Gravity Apparatus. The determination of specific gravity is made by the falling-drop technique. The time that a drop requires to fall through a slightly lighter, immiscible liquid is characteristic of its size and density. The apparatus shown in Figure 16 consists basically of a pipetter and a series of tubes suspended in a clear-plastic, constant-temperature bath.

The analyzed samples are aqueous; therefore the tubes are filled with immiscible organic liquids covering the desired density range. The pipetter is similar in design to the remote pipetter just described but with one tenth the volume capacity.

In practice, after the pipetter is filled with sample, the pipetter tip is lowered under the surface of one of the organic liquids. A 5×10^{-3} ml. drop is delivered, which breaks away from the pipetter tip when it is pulled up and out of the organic liquid. The drop is watched on its downward descent by aid of mirrors. The analyst measures the time required for the drop to fall between two lines scribed on the tube.

The conversion to specific gravity is made by referring to a series of empirical calibration curves. The calibrations are made with a series of aqueous standards whose specific gravities are based on pycnometer measurement.

Acid Titration Apparatus. The third standard box contains apparatus for the determination of acidity by potentiometric titration. The conventional buret of analytical chemistry has been replaced by the liquid end of a Milton-Roy Minipump. The pump plunger is actuated by an air cylinder controlled from a manual key on the shielding face. Each depression of the key delivers 0.1 ml. of standard base from a reservoir mounted inside the cabinet and is recorded on a counter.

As shown in Figure 17, the apparatus also consists of a pneumatic, vertically moving piston which supports a magnetic stirrer and beakers. The glass and calomel electrodes are suspended in a Teflon support and are connected to quick disconnects at the rear of the box. The pH meter is mounted on the shielding face.

To perform a titration, the analyst readies the sample, positions it beneath the electrodes, delivers increments of caustic, and watches the pH meter for the break which indicates that the equivalence point has been passed. The exact end point is determined by a derivative type of calculation.

This equipment has yielded precision data superior to that of conventional buret titrations. However, the development of an improved acid procedure requiring titration to a fixed pH value end point has necessitated modification of this equipment. A microburet is mounted outside the shielding wall and delivers standard caustic to the titration beaker by means of $^1/_8$-inch-diameter polyethylene tubing extending through the shielding.

Solvent Extraction Apparatus. The mass spectrometer method that is used to determine the isotopic distribution of uranium requires high-purity uranium samples. The apparatus in Figure 18 is used for purifying and separating the uranium from foreign ions, including fission products. Liquid-liquid extraction, carried out in test tubes, is used for this purpose.

The test tubes are supported in a circular holder which can both be moved about its axis and raised and lowered. Separate Bodine motors are mounted for this purpose. A stirrer, pipet holder, solvent delivery tubes, and liquid removal tubes are mounted in a second, nonmoving, circular disk positioned above the circular holder.

An aliquot of the sample is delivered by the remote pipetter into a test tube already containing a salting agent solution. The tube is transferred via the sample dolly to the solvent extraction box. Organic extractant is added, the mixture is stirred, and the organic phase containing the uranium, free of foreign ions, is transferred to a clean test tube. This test tube is transferred, via the sample dolly and dumb-waiter, to the upstairs conventional laboratory where the uranium is concentrated prior to its mass analysis.

Fluorophotometric Apparatus. A mock-up of apparatus for the remote determination of trace amounts of uranium by pellet fluorophotometry is shown in Figure 19. This method is complex, involving preparation of sodium fluoride pellets in

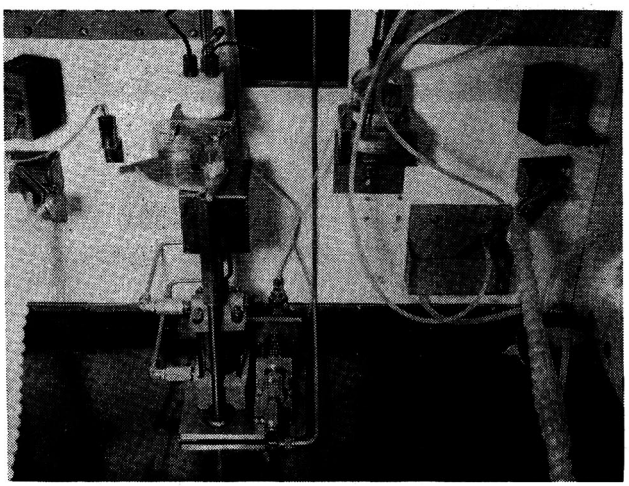

Figure 17. Potentiometric acid apparatus

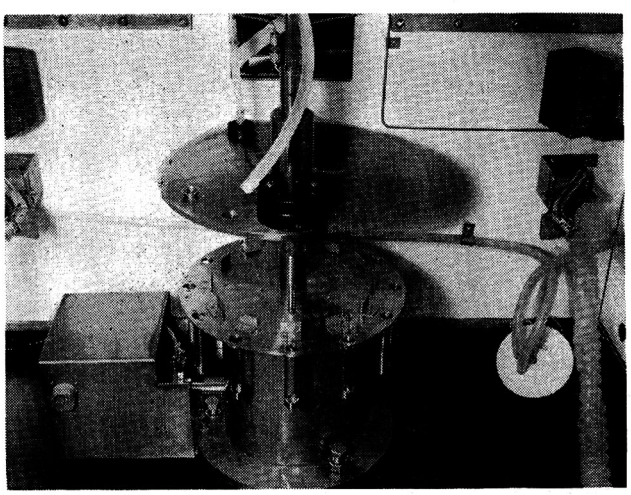

Figure 18. Solvent extraction apparatus

shallow platinum plates, delivery of an accurate volume aliquot of the sample, separation of the uranium from foreign ions by solvent-solvent extraction, delivery of a measured volume of the separated uranium onto the pellet, fusion of the pellet, and measurement of the fluorescence emitted by the pellet under ultraviolet excitation.

The last four steps are carried out on the unit. Ceramic plate holders, containing plates of pellets, are fed onto a chain conveyor. The conveyor moves with intermittent dwell periods. An aliquot of the separated uranium, obtained from a liquid-liquid extraction of the sample previously made in the box, is pipetted onto the pellets. The plates pass under a tubular dryer, then through the field of an induction heater work coil where the pellets are fused twice in a nitrogen atmosphere. The fused pellets then travel under the reader of a modified Galvanek-Morrison fluorimeter whose output is fed to a strip chart recorder. As the plate holders emerge from the reader, the plates are separated. Both are cleaned and transferred to the upstairs laboratory where they are re-used.

Until this apparatus can be completed, trace amounts of uranium are determined by carrying out a liquid-liquid extraction of a sample aliquot. The uranium-containing organic phase, if below activity tolerance, is transferred to the open laboratory for a conventional fluorophotometric uranium analysis. The remote apparatus used is similar to that used for the decontamination of samples prior to mass analysis.

Solid Waste Removal. A special box is provided at the end of each analytical line for the removal of solid waste material. This material consists of expended sample bottles, analytical glassware, and any solid matter from boxes that should go to waste. The material is placed in ice cream cartons and transferred via the sample dolly to this special waste box.

A shielded pig with a capacity of twelve, 1-quart ice cream cartons is supported on a narrow railway in this box. The cartons are transferred from the sample dolly to the shielded pig with a manipulator. As shown in Figure 20, whenever the pig must be emptied, a special dolly is brought to the end of the line on the box dolly tracks, lead doors are manually opened, and the shielded pig is pulled out of the waste box. The dolly and pig are moved to the decontamination area where the waste is stored until eventual transfer to a burial area.

CONVENTIONAL LABORATORY

The conventional laboratory, shown in Figure 4, is immediately above the remote laboratory and covers an area 27 by 73 feet. It serves the dual purpose of providing space and apparatus for the analysis of noncontaminated samples and the carrying out of auxiliary functions as required for the operation of the remote laboratory.

The laboratory contains about 140 feet of open benches, two glassware sinks, one hand sink, three stainless steel hoods, one special hood for perchloric acid fuming, and two small offices. The dumb-waiters that connect this laboratory to the remote lines terminate in still another hood, 16 feet in length, shown in Figure 21. A sink is located in the middle of this hood; the dumb-waiters are at both ends. This hood serves to confine possibly contaminated material coming from the remote lines to an enclosed area. Special glassware can be washed and re-used. Fixed radiation detectors continuously monitor the radiation level. Items are also checked by portable counters before their transfer to the open room.

Miscellaneous fixtures of the laboratory include an under-the-bench refrigerator, a 500-pound-capacity dumb-waiter, an intercommunications system including the remote laboratory, and an extensive compressed gas system. The dumb-waiter connects to the main corridor below and is used for transfer of reagent solutions and other heavy material from the main plant. Nitrogen, oxygen, hydrogen, methane, and hydrogen sulfide are normally supplied from cylinders located in a special storage bay off the first floor main corridor.

DECONTAMINATION AREA

The decontamination area, adjacent to the remote laboratory, is about 25 × 75 feet in over-all area. It features a depressed stainless steel floor, 48 × 25 feet, shown in Figure 22. Here are installed such decontamination equipment as a sand blaster, shoe buffer, spray and steam box, soaking tank, scrub tray and conventional hoods. The remaining area of the room is for temporary solid waste storage except for a portion of the wall adjoining the remote laboratory where box mock-up facilities are installed. The mock-up facilities include two bases for analytical boxes and utility services. One base is equipped with jigs and gages, so that boxes may be checked for dimensional tolerance before transfer to the remote laboratory. The other base is used to support a box for installation of analytical equipment. A manually operated overhead crane is provided for the transfer of heavy objects about the room.

COST

The cost of the entire building and equipment was $2,027,000. Of this amount, $1,755,000 was spent for construction, and $272,000 for design and inspection. The portion of this cost attributed to the analytical laboratories described in this paper was $808,000, of which $220,000 was the basic building cost. For the analytical laboratories the basic building cost per square

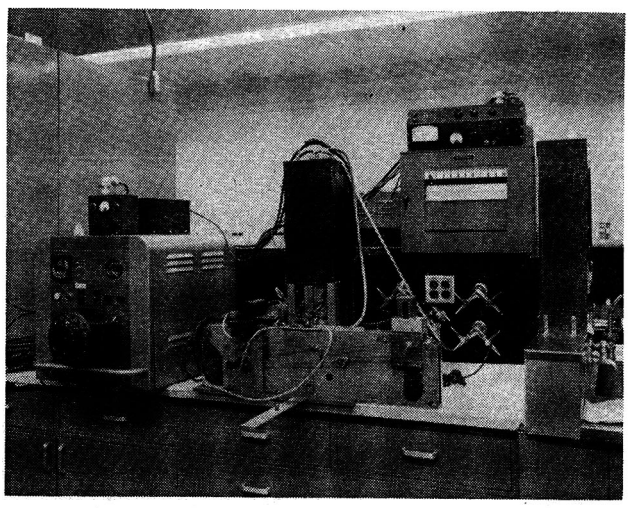

Figure 19. Mock-up of fluorophotometric uranium apparatus

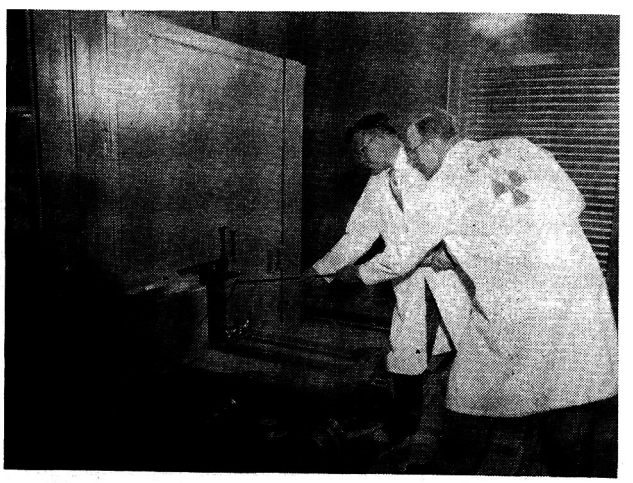

Figure 20. Equipment for disposal of solid waste

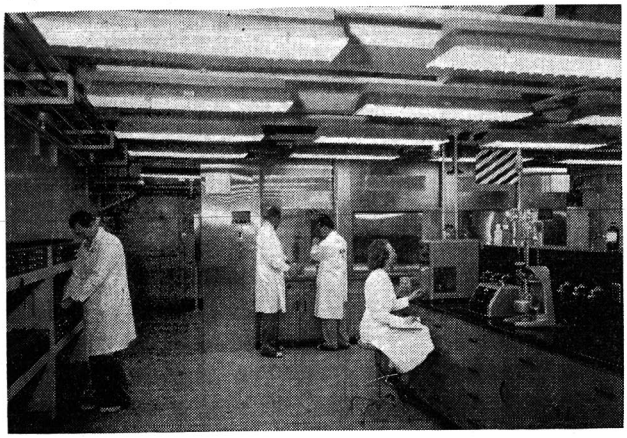

Figure 21. Dumb-waiter and contaminated apparatus hood

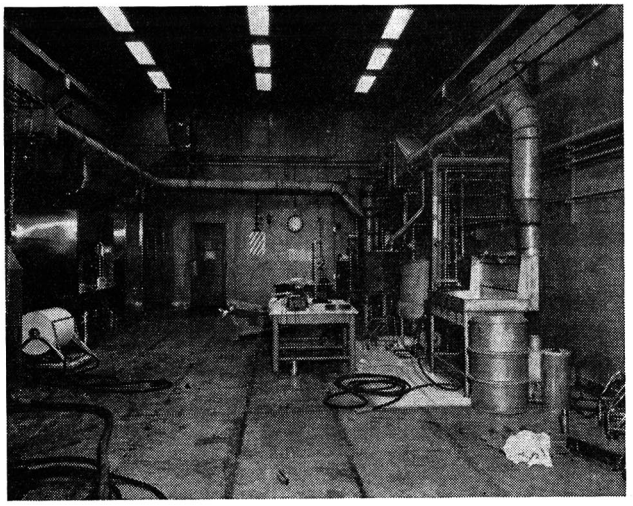

Figure 22. Decontamination room

foot of usable floor space was $80. The total cost per square foot of usable floor space for the same area was $210.

ACKNOWLEDGMENT

The successful completion of a project of this magnitude would have been impossible without the full cooperation of many of the Chemical Processing Plant personnel.

The authors especially acknowledge aid given by the following: Engineering Design Section, Instruments Section, Operations Section, Maintenance Section, Shift Control Laboratory, and the Central Facilities Shops.

Final credit is due the Blaw-Knox Co., Pittsburgh, Pa., for building design and construction supervision, the J. F. Pritchard Construction Co., Kansas City, Mo., for building construction, and the Engineering Branch, Idaho Operations Office, Atomic Energy Commission, for over-all supervision and liaison.

RECEIVED for review September 6, 1955. Accepted February 13, 1956. Presented before the Nuclear Engineering and Science Congress, Cleveland, Ohio, December 12 to 16, 1955. Work performed under contract No. AT(10-1)-205 with the Idaho Operations Office, U. S. Atomic Energy Commission.

Use of Paper Chromatography for Differential Analysis of Phosphate Mixtures

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Several procedures for differential analysis of mixtures of condensed phosphates were developed on the basis of Ebel's technique of ascending paper chromatography. A new quantitative method has been developed for mixtures containing ortho-, pyro-, and triphosphates (tripolyphosphates), as well as rings (trimeta- plus tetrametaphosphates), and long-chain phosphates. In addition to a one-dimensional chromatogram, this technique involves a two-dimensional chromatogram in which the second solvent advances in the opposite direction of the first solvent (180° apart). This procedure and the Ebel technique, in which the solvents are run at right angles to each other, have been put on a routine basis suitable for use in control laboratories. A special colorimetric procedure employing an extraction step makes possible the rapid quantitative evaluation of the chromatographic fractions. Analyses are described for commercial sodium triphosphate, built detergents without the use of any previous treatment of the sample, and surface waters. Hydrolysis during the chromatographic run was investigated and small correction factors are recommended for the most precise results.

THOUGH excellent contributions have recently been made to the general analytical problem of phosphate mixtures (*2, 4, 6, 9, 11, 13, 17, 18, 20, 21*), there is still a need for a reasonably quick procedure which uses easily available equipment and reagents and is sufficiently accurate for a quantitative assay of the commercial phosphates and phosphate-containing products. Paper chromatography for the differential analysis of inorganic phosphorus compounds has been primarily developed by two groups of investigators—one in Canada and one in France. The Canadian investigators have worked out a quantitative procedure (*4, 21*) based on descending chromatography using considerably longer separation times than are employed here. The French group has emphasized qualitative measurements by ascending solvent fronts (*5*). They have also developed a regular two-dimensional technique which separates ring and chain phosphates into two distinct groups (*5, 6*).

The semiquantitative techniques described by Ebel have been developed into a reasonably precise quantitative procedure by the use of sample bands (containing larger sample weights) instead of spots and very short runs to minimize errors due to hydrolysis. The precision of this procedure appears to be as good as that obtained with descending chromatography (*4, 21*); use of a hydrolysis correction improves the accuracy. The

elapsed time for a quantitative assay is considerably shorter than that considered necessary by previous investigators.

These analyses are applicable to mass-production techniques because common glass jars are employed in place of the elaborate and well-sealed chromatographic chambers usually needed for clear-cut separation in descending chromatography (*4*, *21*).

EXPERIMENTAL

Equipment and Materials. Rectangular battery jars, about 12 by 8 by 6 inches.

Cylindrical jars (1-gallon pickle jars), about 6 inches in diameter with 3½-inch opening; about 11 inches high and fitted with Petri dish covers.

Micropipets, 50 μl. with subdivisions for 10 μl.

Screw control for micropipets.

Platinum wire, about 0.02 inch in diameter.

Chromatographic spray bottle.

Ultraviolet lamp, long wave, which can be arranged to cover an area 9 by 9 inches.

Water bath, consisting of a hot plate with several 600-ml. glass beakers and glass beads.

Safety aspirator, such as a Propipette from Will Corp.

Electrophotometer, with a light path of 10 to 20 mm., and a red filter with its maximum at 620 to 650 mμ.

Filter Paper, Schleicher & Schuell, 589, orange ribbon, special paper for paper chromatography.

Reagents. EBEL'S CHROMATOGRAPHIC SOLVENTS (*5*, *6*). Acid solvent is made by mixing 750 ml. of isopropyl alcohol, a solution of 50 grams of trichloroacetic acid in 250 ml. of water, and 2.5 ml. of concentrated ammonia. Basic solvent is made by mixing 400 ml. of isopropyl alcohol, 200 ml. of isobutyl alcohol, 390 ml. of water, and 10 ml. of concentrated ammonia.

CHROMATOGRAPHIC SPRAY. This is made according to Hanes and Isherwood (*10*) by mixing 5 ml. of 60% perchloric acid, 1 ml. of concentrated hydrochloric acid, and 1 gram of ammonium heptamolybdate tetrahydrate and diluting to 100 ml. with distilled water.

REAGENTS FOR EXTRACTION AND HYDROLYSIS. Ammonia and sulfuric acid, both about 8*N*.

REAGENTS FOR COLORIMETRIC DETERMINATION OF PHOSPHORUS PENTOXIDE. The following are required: a 10% aqueous solution of ammonium heptamolybdate tetrahydrate; a mixture of equal volumes of isobutyl alcohol and benzene; 2% sulfuric acid (by volume) in aldehyde-free absolute methanol; a solution of 25% stannous chloride in concentrated hydrochloric acid, prepared according to Martin and Doty (*12*); 1*N* sulfuric acid; and reducing agent. This latter solution is not stable for more than 1 day and is prepared as needed by adding 0.5 ml. of stannous chloride solution to 100 ml. of 1*N* sulfuric acid.

Reference Standards. Reagent grade chemicals were used to prepare the standards listed below, except for sodium triphosphate hexahydrate, a laboratory preparation purified by fractional recrystallizations (*17*); sodium trimetaphosphate, a laboratory preparation purified by two recrystallizations and dried at 110° C. (*14*); and sodium tetrametaphosphate tetrahydrate, a laboratory preparation (*8* or *1*) followed by recrystallization.

PYROPHOSPHATE STANDARD, (*A*). This contained about 1 γ of phosphorus per μl., and was made by dissolving about 0.7 gram of tetrasodium pyrophosphate decahydrate in 100 ml. of water.

ORTHO-, PYRO-, TRI-, TRIMETAPHOSPHATE STANDARD, (*B*). This standard contained about 0.4 γ of each phosphorus species per μl.—i.e., about 1.6 γ of total phosphorus per μl. It was prepared by dissolving 0.4 gram of monopotassium orthophosphate, 0.7 gram of tetrasodium pyrophosphate decahydrate, 0.5 gram of sodium triphosphate hexahydrate, and 0.35 gram of sodium trimetaphosphate [$(NaPO_3)_3$] in 250 ml. of water.

ORTHO-, PYRO-, TRI-, TRIMETA-, TETRAMETAPHOSPHATE STANDARD, (*C*). This standard contained about 0.4 γ of each phosphorus species per μl., or about 2.0 γ of total phosphorus per μl. It was prepared like standard *B*, except that 0.4 gram of sodium tetrametaphosphate tetrahydrate [$(NaPO_3)_4 \cdot 4H_2O$] was added.

General Technique. SAMPLE WEIGHT. The capacity of the chromatographic paper for short runs is 10 γ of total phosphorus per spot. The most convenient volume for one spot is about 5 μl., which yields spots on the paper about ⅜ inch in diameter. The sample solution should therefore contain 2 γ of total phosphorus or a little less per μl. More dilute sample solutions can be used by repeatedly applying 5-μl. droplets on the same spot, with complete drying after each addition. In qualitative analysis, the point of the micropipet must touch the paper exactly on the center mark of the sample spot each time. Up to 15 subsequent applications may be carried out without destroying the texture of the chromatographic paper. This means that volumes up to 0.075 ml. can be applied on one spot, without exceeding the convenient diameter of the sample spot of about ⅜ inch.

PRETREATMENT OF CHROMATOGRAPHIC SOLVENTS. Because better patterns are obtained with solvent that has been used in a couple of runs, a pretreatment of the solvent is carried out by simulating two runs, using corresponding areas of the chromatographic paper and following the procedure described later. The solvent can be used by replenishing the volume after every three or four runs with fresh untreated solvent.

PROCEDURE. The filter paper is cut into sheets 9 inches wide by 6 or 9 inches high, depending on the analysis for which it is being prepared. Contamination, even with traces of phosphates, must be avoided. The starting lines and spots for sample and reference material are marked, as indicated later, with an ordinary graphite pencil, and the sheets are stored lying flat in a dust-free cabinet.

Immediately before the chromatographic analysis is started, the prepared sheet is folded into a cylinder and clipped together with platinum wire about 1½ inches long, in such a way that the edges do not touch. The sample solution and the reference solution are applied on the proper starting spots with the micropipet, and the spots are allowed to dry for a few minutes.

The cylinder is then inserted into a rectangular battery jar which contains a beaker with some of the chromatographic solvent to be used in the subsequent separation, and the jar is covered with a well-fitted glass plate. If more than one sheet is stored in the jar at the same time, the sample spots on one sheet must not touch the other sheet. The cylindrically rolled sheet is exposed to the vapor for 45 minutes, without getting drops or spots of the solvent on the paper.

After this pretreatment the paper cylinder is transferred without delay, starting line or starting spot down, to a cylindrical jar (1-gallon) which contains 150 ml. of the chromatographic solvent. This jar is covered immediately with the fitted Petri dish. The sheet is inserted carefully to avoid splashing or wave-like movement of the solvent which would cause an uneven starting level for the ascending run. The upright edges must not touch each other. The 150-ml. volume of chromatographic solvent in the jar forms a layer about ⅜ inch high; the sample and reference spots, which in all cases are applied 1 inch above the bottom edge of the sheet, are thus not immersed in the solvent. This prevents washing out of the sample and contamination of the chromatographic solvent. The jar must not be moved, and must be kept covered during the run.

When the run is complete, the sheet is easily removed by grasping the two upper corners and folding them slightly over each other so that the cylinder is deformed to a cone. The excess chromatographic solvent is removed from the paper by touching the bottom of the upright cylinder to a paper towel or absorbent tissue several times.

The sheet cylinder is left standing on the towel for about 10 minutes, then transferred to a drying oven at 50° C. for 10 more minutes. The platinum clip is removed, the sheet is bent flat and sprayed evenly over the whole area with the chromatographic spray solution. Drops of liquid must not form on the paper. The sprayed sheet is dried for 10 minutes in the oven at 50° C., then placed under the ultraviolet lamp and irradiated until the blue zones appear. If the sheet has been covered evenly by the irradiation, this operation is complete after the reference spots are clearly visible.

The zones should be marked with pencil soon after they have become visible in such a way as to exclude reference spots definitely but to include the entire paper area which carries the respective fraction. Moderately curved lines are as satisfactory as straight lines. If two fractions of the pattern are located close together, the pencil mark must follow the line of the weakest color intensity. When band chromatograms obtained in an acidic one-directional run are analyzed, the separating line between the pyro- and triphosphate fractions is drawn as close as possible to the pyro band, because the correction value (given later) was determined empirically under these conditions. The ortho fraction is marked symmetrically to the colored zone and any paper area left over between the pyro and ortho bands can be discarded (only minute amounts of phosphorus which fall within the error of the colorimetric determination are located on this area after a 2½-hour run). In a two-directional band chromatogram for ring phosphates the line which separates rings from chains is drawn half-way between the ring band and the chain bands. If a fraction is too weak to show up, an area is marked in the position indicated by the reference spots.

For the short-cut elution (described later) it is essential to match the paper areas within ¼ square inch or better. An area of about 3 square inches of the phosphate-free blank area is also marked on every sheet for quantitative analysis. This area is treated like the other fractions to obtain a reagent blank.

Each zone is cut individually into about 1/8-inch strips without losing any small clippings. These strips are coiled irregularly and inserted fairly tightly into small funnels with tapered stems of a size that fits easily into the necks of 25-ml. volumetric flasks. A piece of bent platinum wire prevents air-tight sealing. The paper in the funnel is moistened with a few drops of distilled water and pushed down with a small bent spatula in such a way that the paper forms an extraction column. The flow rate is adjusted to about 1 drop per minute by loosening or pushing down the paper with the spatula, which is rinsed afterwards with a few drops of distilled water. The elution is started by adding 4 drops of 8*N* ammonia; as soon as the ammonia has penetrated the paper, 4 drops of distilled water is added. The ammonia-water treatment is repeated three times followed by two washes with several drops of distilled water. Then four more wash cycles are carried out using 8*N* sulfuric acid instead of ammonia. Medicine droppers which deliver practically equal volumes of ammonia and sulfuric acid aid in obtaining a nearly neutral eluate. The paper column is rinsed five additional times with several drops of distilled water, and the funnel is removed. Then 2 ml. of 8*N* sulfuric acid is added to the eluate, which is then ready for hydrolysis.

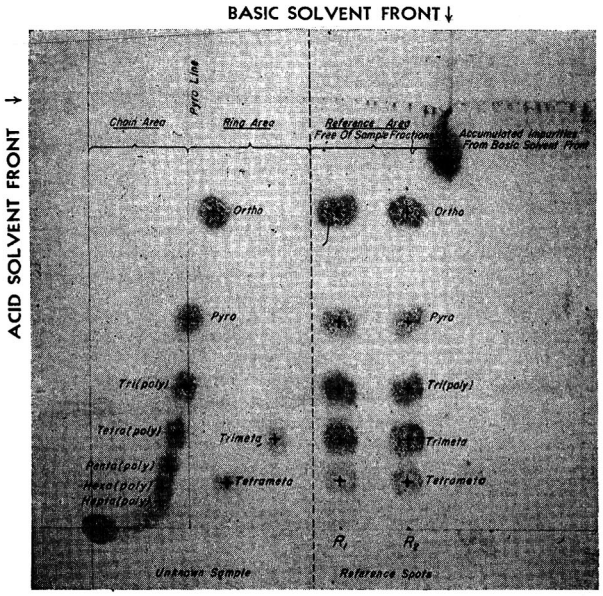

Figure 1. Two-directional chromatogram for qualitative identification of ring and chain phosphates

Short-Cut Procedure without Elution Step. The paper areas for the single fractions are matched to within 1/4 square inch, using some paper from the paper blank area if necessary. Wide zones can be handled as two or three fractions. Each fraction, as well as a 3-square inch reagent blank, is cut into pieces (about 1/2 inch long and 1/8 inch wide) and transferred to 25-ml. flasks. After 1 ml. of 8*N* ammonia and 7 ml. of distilled water have been added, the flasks are swirled and allowed to stand for 5 to 10 minutes. Then 3 ml. of 8*N* sulfuric acid is added—1 ml. to be equivalent to the ammonia already added and 2 ml. to make the solution strongly acidic for hydrolysis. All the flasks containing the fractions of one analysis must be handled as a group to ensure similar treatment.

Hydrolysis and Color Development. The 25-ml. flasks, containing all the phosphate in a strong acidic solution, are placed in a water bath at the boil for 20 minutes. Though the orthophosphate fraction does not require any hydrolysis, it is included in the procedure to ensure uniform treatment. This 20-minute period is sufficient to hydrolyze all condensed phosphates present in this solution.

After the flasks have cooled, exactly 10 ml. of the benzene–isobutyl alcohol mixture is added to each, followed by 2 ml. of 10% ammonium molybdate solution. The volume is made up to the 25-ml. mark with distilled water and the phosphomolybdate complex is extracted by vigorous shaking for at least 20 seconds. After the layers have separated, exactly 5 ml. of the supernatant organic layer is transferred into another 25-ml. volumetric flask and diluted with about 10 ml. of the sulfuric acid–methanol. Then 1 ml. of reducing agent is pipetted into the flask. The contents are mixed and the volume is made up to the mark with sulfuric acid–methanol. The blue solution is carefully homogenized by shaking. After 10 minutes the absorbances are read against distilled water on an electrophotometer at about 650 mμ (red filter). The cells should have a light path of 10 to 20 mm. They must be small enough to permit efficient rinsing with relatively small volumes, because the colored solution has a volume of only 25 ml.

The absorbance of the reagent blank is deducted from the other readings to give a net absorbance for every fraction. The total absorbance for the analysis is determined by adding the net absorbances of all the fractions. The per cent phosphorus for every fraction of the total phosphorus present is determined by multiplying the net absorbance for the fraction by 100 and dividing by the total absorbance for the respective analysis.

SPECIFIC APPLICATIONS

Qualitative Identification of Ring and Short-Chain Phosphates. In the qualitative two-directional separation described by Ebel (*5, 6*) the R_f values were found to vary considerably with the experimental conditions, especially in the basic solvent. As a result, positive qualitative identification of unknowns is not always possible if the conditions are not controlled precisely. This difficulty has been solved by adding pyrophosphate as an internal standard, and using a mixture of readily-available known phosphates as comparison standards in the acid run. Pyrophosphate as the internal standard reproducibly established the borderline between the ring-plus-orthophosphate and the chain-phosphate areas. The comparison standards are applied after the basic run to the area which is free of unknowns but has been penetrated by the basic solvent. The R_f values of ortho-, pyro-, and triphosphate are obtained from the comparison standards on the same sheet and are used to prepare a calibration curve for identification of higher chain phosphates. This technique has been used to identify ion exchange fractions up to heptaphosphate from a glassy sodium phosphate and has proved successful in assaying condensed phsophates in a wide variety of mixtures with different substances.

Procedure. For every sample two sheets (9 by 9 inches) are marked with two lines 1 inch from two adjoining edges. The crossing point of these lines is the starting point in one corner. Each sheet is coiled into a cylinder and fastened with a platinum clip. A 2-μl. spot of reference standard *A*, which contains only pyrophosphate, is placed exactly on the starting point of one sheet. This sheet is dried, and then 5-μl. drops of the sample solution are added on the starting points of each of the two sheets. The sheets are run in basic solvent for 8 to 9 hours, during which time the phosphate species migrate at their respective speeds along the pencil line parallel to the upright edge, which becomes the starting line for the second run. After the paper is dried, the platinum clip is removed, and the cylinder is unfolded. The height of the solvent front is measured, and the upper third of that portion of the sheet which has been penetrated by the solvent is marked as reference area. In this area, one or two reference spots are marked 1 inch apart on the starting line for the second run. The sheet is coiled into a cylinder again, in such a way that the starting line for the second run is located at the bottom of the cylinder. Droplets of 5 μl. of reference standard *C* are put on the marked reference spots. A separation in acid solvent is run for 8 to 9 hours, and the pattern is developed. A photograph of such a developed pattern is shown in Figure 1. (The spot marked as "accumulated impurities from basic solvent front" consists of fluorescent material and shows up highly exaggerated in the figure.)

Qualitative Evaluation. On the sheet containing reference standard *A*, a line is drawn through the center of the pyro spot (Figure 1). The ring and chain phosphates are identified by the aid of the reference spots; their locations are clearly shown in Figure 1.

Tetraphosphate is found very close to the trimeta level and pentaphosphate near the tetrameta level. For the positive identification of the slower moving hexa- and heptaphosphates, a calibration curve is prepared by plotting the logarithms of the R_f values—i.e., (distance moved by spot in acid solvent)/(distance moved by acid-solvent front) *vs.* the chain length for the ortho-, pyro-, and triphosphate—as found from the reference area. From an extrapolation of this line, the numbers of phosphorus atoms corresponding to the logarithms of the R_f values of the unknown species are determined. The second sheet is required for recogniz-

ing any pyrophosphate to be found in the sample itself and for eliminating errors from incidental contamination such as fingerprints.

Typical R_f values obtained on solutions containing a mixture of phosphates are shown in Table I. The two sets of R_f values given for the acid solvent illustrate the variations to be expected from one experiment to another. A plot of log R_f *vs.* n_c for the chain-phosphates in the acid solvent gives a straight line, as found by Grunze and Thilo (*9*).

SEMIQUANTITATIVE EVALUATION. The spots are cut out and analyzed according to the general procedure for quantitative evaluation. Because the sample weight is in the range of only 10 γ of phosphorus, the reading errors of the colorimetric procedure give deviations of several per cent.

Quantitative Assay of Commercial Alkali Triphosphates. A sample of 1.5 grams is dissolved in 250 ml. of distilled water and well mixed. By one-directional chromatography in acid solvent, percentages for ortho-, pyro-, and long-chain phosphate are obtained. The analysis for ring phosphate is separately accomplished by running a two-directional separation, first in basic solvent then in acid solvent.

ONE-DIRECTIONAL RUN. A sheet 9 by 6 inches is laid out into the areas for blank and sample (the solid pencilled lines in Figure 2). Droplets of 5 μl. of the sample solution are applied on each x mark on the starting line; 5-μl. droplets of reference standard B are applied at R_1 and R_2.

Each half of the sheet represents one analysis with a common blank area of paper between. The chromatogram is run in acid solvent for 2 1/2 hours at room temperature. The triphosphate fraction is cut to include any trimetaphosphate present, as indicated by the trimetaphosphate reference spots (next to the starting line on Figure 2). The quantitative analysis of the

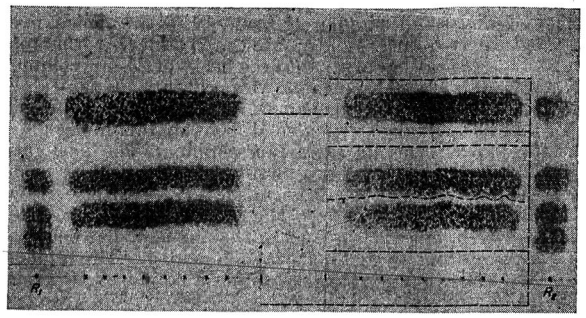

Figure 2. Pattern showing ortho-, pyro-, and triphosphate on typical sheet for one-directional quantitative run

Right half marked with dotted cutting lines

chromatogram is carried out as described previously. To correct for hydrolysis of triphosphate on the paper during the run, 0.3% of the phosphorus found as triphosphate is added for each hour of the run. The same figure is deducted from the percentage found for the pyro fraction to correct for the hydrolysis products having migrated into this area.

TWO-DIRECTIONAL RUN. A sheet 9 by 6 inches is prepared and marked as shown in Figure 3. Droplets of 5 μl. of sample solution and of reference standard B are placed at x and at R_1, R_2, and R_3, respectively. The chromatogram is run in basic solvent at 25° C. for 4 to 5 hours. The sheet is removed from the jar, dried, bent flat, and the strip carrying the reference spot R_1 is cut off along the dashed line in Figure 3. The reference strip is sprayed and developed. By the aid of the reference strip, the upper level of the highest phosphate fraction (trimetaphosphate) is marked on the unsprayed main sheet. The sheet is then cut 3/4 inch above this level, coiled into the cylinder again, and inserted upside down without any pretreatment period for the acid solvent run, which is extended over 1 1/2 hours. When the pattern is developed, the trimeta area appears as a band, clearly separated from the other phosphates and located near the edge of the sheet cut off prior to the second run. The percentage of total phosphorus as ring phosphate is determined as previously described.

RESULTS. Typical data found in using this procedure are summarized in Table II. The first sample analyzed was a mixture of highly purified alkali ortho-, pyro-, and triphosphates. The results of four determinations show that the accuracy and reproducibility of this chromatographic procedure are always better than 1% of the total phosphorus. The second analysis in

Table I. R_f Values of Phosphates in Basic and Acidic Solvents

(Room temperature)

Phosphate Species	Chain Length (n_c)	R_f Basic solvent	R_f Acid Solvent	
Orthophosphate	1	0.28	0.68	0.73
Pyrophosphate	2	0.20	0.42	0.48
Triphosphate	3	0.18	0.21	0.33
Tetraphosphate	4	0.15	0.11	0.22
Pentaphosphate	5	0.13	0.06	0.14
Hexaphosphate	6			0.09
Heptaphosphate	7	0.11	0.02	0.06
Trimetaphosphate	..	0.35	0.14	0.21
Tetrametaphosphate	..	0.26	0.08	0.11

Table II. Results of Differential Analyses of Phosphates

Sample	Phosphate Species	% Phosphorus Present	Found I	II	III	IV
Pure phosphate mixture[a]	Orthophosphate	35.03	34.4	35.5	35.4	34.8
	Pyrophosphate	35.43	35.7	34.7	35.4	35.4
	Triphosphate	29.53	29.9	29.8	29.2	29.8
Sodium triphosphate hexahydrate, recrystallized[b]	Orthophosphate	0.6	1.0	1.0		
	Pyrophosphate	1.2	1.4	1.5		
	Triphosphate	98.2	97.6	97.5		
Commercial sodium triphosphate, high temperature rise	Orthophosphate		0.0	0.0		
	Pyrophosphate		3.8	3.7		
	Triphosphate		94.0	94.0		
	Long-chain phosphate		0.8	0.8		
	Trimetaphosphate				1.4	1.5
Commercial sodium triphosphate, low temperature rise[c]	Orthophosphate		0.8	0.9		
	Pyrophosphate		10.9	10.4		
	Tri- trimetaphosphate		87.2	87.2		
	Long-chain phosphate		1.1	1.4		
Experimental sodium triphosphate	Orthophosphate		0.3	0.15		
	Pyrophosphate		3.2	3.5		
	Triphosphate		96.5	96.35		
	Long-chain phosphate		0.0	0.0		
	Trimetaphosphate				0.0	
Experimental sodium triphosphate	Orthophosphate		0.3	0.3		
	Pyrophosphate		4.5	4.7		
	Triphosphate		94.2	94.0		
	Long-chain phosphate		0.3	0.3		
	Trimetaphosphate				0.7	

[a] The ortho and pyro content of the $Na_5P_3O_{10}.6H_2O$ and $Na_4P_2O_7$ used as starting materials were determined by ion exchange technique (*14*). The figures given in column 3 are calculated from the weights and exact assay of the starting materials.

[b] Figures in column 3 were obtained by ion exchange (*14*); Beukenkamp, Rieman, and Lindenbaum (*8*) also describe an ion exchange procedure.

[c] Not analyzed for trimetaphosphate.

Table II demonstrates that the paper chromatographic procedure gives the same results as chromatography in an ion exchange column. The other analyses are examples of the application of this method to commercial and experimental samples of sodium triphosphate.

Triphosphate and trimetaphosphate cannot be separated in a short one-directional chromatographic run in the acidic solvent. The triphosphate fraction always shows some trailing effect which interferes with a clear separation even in a 5-hour run.

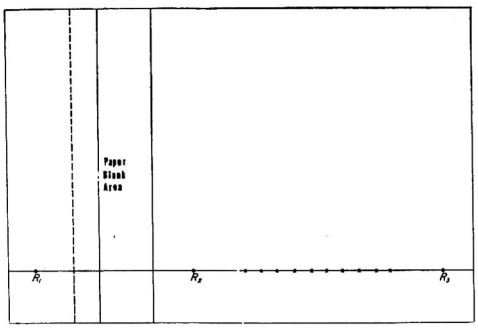

Figure 3. Sheet for quantitative determination of ring phosphates by two-directional separation of sample bands

All trimetaphosphate is definitely included in the triphosphate area if this zone is cut just below the level of the triphosphate-trimetaphosphate reference spot pair at the margin of the sheet (Figure 2). Any tetraphosphate present would also fall on this area. If a short-chain glass is being assayed by this procedure, it must be kept in mind that (1) tetraphosphate as well as trimetaphosphate is included in the value for triphosphate, and (2) pentaphosphate as well as tetrametaphosphate will show up in both the triphosphate and the nonmoving fraction (hexaphosphate and longer chains). The correct way to analyze for those phosphate species is to use extended separations, in which hydrolysis correction values are employed for each species.

Results of the two-dimensional procedure for the determination of ring phosphates are shown in Table III. The solutions were obtained by diluting a trimetaphosphate standard, which was found to contain 90.5% phosphorus as trimetaphosphate by chromatographic analysis, with varying amounts of triphosphate, free of any trimetaphosphate. If more than a trace of nonmoving, long-chain phosphate is found in the one-directional chromatographic run, the whole paper area down to the starting area must be included for the determination of total phosphorus in the two-directional chromatogram. In this case, it is recommended not to let the solvent front in the basic run rise higher than 5 inches above the starting line. This brings the short-chain phosphates in the second run very close to the starting line of the first run, back to the area where the long-chain phosphates are located. Any tetrametaphosphate present migrates slower than trimetaphosphate in the basic run, and also slightly slower than the trimetaphosphate in the acid run. Because these two runs are in opposite directions, both ring phosphates are located in the same area after the two runs. Separation could be accomplished by running a third separation in acid solvent after cutting off the chain phosphate fractions.

Table III. Determination of Trimetaphosphate in Presence of Triphosphate and Minor Amounts of Ortho- and Pyrophosphate

% Phosphorus as Trimetaphosphate	
Calcd. from composition of materials used	Found
51.4	52.0
27.6	27.0
14.3	14.3
7.3	7.1
3.7	3.7
1.9	2.4
0.95	1.2

Table IV. Differential Phosphate Analyses of Detergent Formulations

	Detergent Formulation, %	Phosphate Species	% Phosphorus of Total Phosphorus: Phosphates alone	In detergent mixture	
I[a]	Sodium triphosphate hexahydrate, 32.3; tetrasodium pyrophosphate, 12.4; sodium carbonate monohydrate, 12.3; sodium silicate, No. 9[b], 38.5; Sterox CD[c], 4.5.	Orthophosphate	0.4	0.2	
		Pyrophosphate	31.5	31.4	
		Triphosphate	67.5	68.0	
		Long-chain phosphate	0.5	0.4	
II[a]	Sodium triphosphate hexahydrate, 31.1; tetrasodium pyrophosphate, 16.1; sodium sulfate, 12.3; sodium silicate, No. 9[b], 20.5; Santomerse No. 3[c], 17.7; minor ingredients (including brightening agent and sodium carboxymethylcellulose), 2.3.	Orthophosphate	0.4	0.4	0.4
		Pyrophosphate	38.1	37.8	36.9
		Triphosphate	60.9	61.3	62.3
		Long-chain phosphate	0.6	0.4	0.4
III[d]	Sodium triphosphate, 40; sodium carbonate, 25; sodium silicate, N Brand[e], 20; Sterox CD[c], 14; brightening agent plus perfume plus sodium carboxymethylcellulose, 1.	Orthophosphate	0.2	0.5	0.4
		Pyrophosphate	7.2	7.5	7.3
		Triphosphate	92.6	92.0	92.3
		Long-chain phosphate	0.2	0.0	0.0
IV[d,f]	Sodium triphosphate, 20; tetrasodium pyrophosphate, 20; sodium carbonate, 25; sodium silicate, N Brand[e], 20; Sterox CD[c], 14; brightening agent plus perfume plus sodium carboxymethylcellulose, 1.	Orthophosphate	0.2	0.9	0.9
		Pyrophosphate	51.7	50.8	51.4
		Triphosphate	47.7	48.0	47.6
		Long-chain phosphate	0.2	0.2	0.0

[a] Ortho and pyro content of the $Na_5P_3O_{10}.6H_2O$ and $Na_4P_2O_7$ used as starting materials were determined by ion exchange technique (*15*). Long-chain phosphate content of the $Na_4P_2O_7$ was determined by paper chromatography. Figures given under "Phosphates alone" are calculated from weights and assays of starting materials.
[b] E. I. du Pont de Nemours & Co.
[c] Monsanto Chemical Co.
[d] Sodium triphosphate used as starting material was assayed by paper chromatographic technique.
[e] Philadelphia Quartz Co.
[f] Sample of pyrophosphate used as starting material not assayed for ortho content. High value for ortho found in detergent mixture indicates that pyrophosphate contained a small amount of orthophosphate.

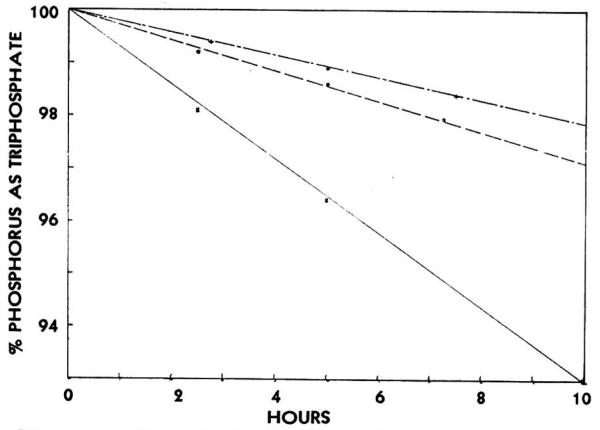

Figure 4. Hydrolysis of triphosphate in acid chromatographic solvent at various temperatures

—·—·—·— 22 °C.
– – – – – – 25 °C.
———— 30 °C.

Differential Phosphate Analysis of Detergent Mixtures. The assay just described can be used to analyze built detergent compositions for their phosphate content. For such an analysis a solution is prepared by dissolving 3 grams of material in 250 ml. of distilled water. The total phosphorus present is determined colorimetrically after diluting an aliquot in the ratio of 5 to 1000 and carrying out a complete hydrolysis. The chromatographic analysis is carried out as described for the assay of sodium triphosphate. If the detergent contains less than 5% total phosphorus, the application of sample solution is repeated after complete drying of the first series of sample spots.

Various detergent additives were tested and found not to interfere with the separation and quantitative determination of the phosphate species. This freedom from interference is attributable in great part to the solvent extraction used in the colorimetric analysis. The results of the analyses of several different detergent compositions are summarized in Table IV. This technique should prove to be of great value in studying the problems associated with the degradation of condensed phosphates in the processes of manufacturing built detergents.

Differential Phosphate Analysis in Very Dilute Solutions—e.g. Surface Waters. A convenient aliquot of a pure neutral solution of alkali phosphates which contains about 50 to 100 γ of total phosphorus (determined colorimetrically) is allowed to evaporate at room temperature. Aliquots of 10 ml. will evaporate in Petri dishes of 4-inch diameter at 25° C. overnight; this can be shortened by moving a stream of filtered air over the surface. The residue is taken up with 0.25 ml. of distilled water, which is spread and then collected again on one side of the slightly tilted dish with the aid of a rubber stopper. The dish is supported in the slanted position and the solution is applied to the chromatographic paper on both halves of the sheet. The analysis is carried out like a triphosphate assay and the hydrolysis correction is applied.

In Surface Water. A sample of 100 ml. is stirred for 1 hour with 1 gram of Dowex-50 resin, sodium form. After filtration through a Whatman No. 1 filter (the first 25 ml. is discarded), 10 ml. of the filtrate is transferred to a Petri dish and evaporated. The dry residue is taken up with 0.4 ml. of a 0.5% solution of sodium Versenate and treated as just described, except that the solution is applied in many 2-μl. droplets along the starting line rather than in 5-μl. droplets on the x marks. Sample droplets are also put on the reference spots, R_1 and R_2. The sheet is dried completely before starting a subsequent sample application. Finally droplets of reference standard B are put on the reference spots R (on which droplets of sample solution have already been applied). The separation is run in acid solvent for $3^1/_2$ hours and completed as for the triphosphate assay, including the correction for hydrolysis.

The results of analyses of very dilute phosphate solutions of varying degrees of hardness are given in Table V. These analyses were carried out on freshly prepared dilutions in order to avoid difficulties experienced with the abnormally high rates of hydrolysis from the catalytic action of multivalent cations, clay particles, and microorganisms present in these solutions. The Versenate treatment is included to ensure dissolution of insoluble calcium phosphates that might be present, because the ion exchange treatment does not completely remove calcium from the solution. The effect of Versenate solutions on insoluble calcium phosphates was checked; it was found that these materials can be brought into solution and analyzed by the chromatographic procedure described.

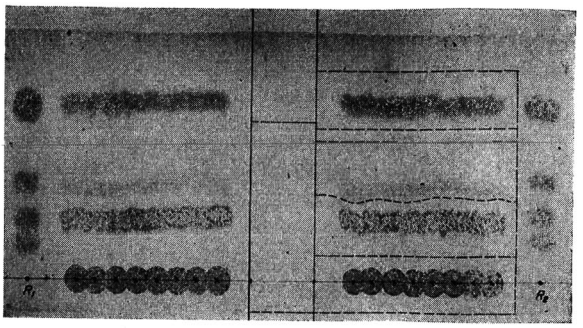

Figure 5. Separation after $2^1/_2$-hour run in acid solvent

Right half marked with dotted cutting lines

DISCUSSION

The applicability of ascending paper chromatography for separating condensed phosphates is limited. In the pH range required for rapid separation of the chain phosphates (about 1.5 to 1.7), the distribution over the available distance as indicated by the R_f values (Table I) is not favorable for high molecular weight phosphates, because more than 90% of the available distance is occupied by the first seven members of the chain-phosphate family. Similar data have been reported by Grunze and Thilo (*9*). By extrapolating these data, it is apparent that less than 1% of the distance traveled by the solvent front is available for all chain phosphates with more than 15 phosphorus atoms—e.g., about 1 cm. if a 250-cm. sheet were used. In descending chromatography, this difficulty can be overcome by letting the fastest moving phosphates flow off the sheet, although continuous examination of the solvent leaving the paper is necessary to determine the number and identity of phosphate fractions. However, hydrolysis of the phosphates and the diffusion of the spots, over the long periods of time required, would establish similar limitations. When basic solvents are used, the distribution of the phosphates is slightly better (Table I), but the separation of chain phosphates proceeds even more slowly, and any trend to uneven solvent flow makes identification and evaluation of the chromatograms extremely difficult. The separation of condensed phosphates exhibiting chains of 10 or more phosphorus atoms remains a problem which is best investigated by ion exchange rather than by paper chromatography. On the other hand, paper chromatography offers an extremely simple scheme for the analysis of mixtures of orthophosphate and the lowest condensed phosphates.

The R_f values of the individual phosphate species in a certain chromatographic solvent are not strictly reproducible. They vary to some extent with the composition of the sample, the texture of the paper, pretreatment of the paper sheet, temperature, duration of the run, and the exact composition of the chromatographic solvent, as well as the number of times the solvent was used previously. Precautions taken in complete sealing of the chromatographic chamber are also important. The R_f values are also influenced by other constituents in the sample, so that pure reference samples sometimes do not indicate the migration rates correctly. This has been found to be particularly important and it has been accounted for in the analysis of surface

waters of high hardness, containing very small amounts of phosphates.

Reference spots should be applied on every sheet. The operation takes only seconds and ensures reliable identification of phosphate species. The reference spots are also used for locating minor phosphate fractions in quantitative runs.

Quantitative evaluation of phosphate patterns by comparing the size and intensity of the blue zones developed on the paper is not feasible, because neither the degree of hydrolysis nor the reduction is complete during formation of the blue zones. The quantitative determination of each fraction is carried out after cutting and eluting the respective paper areas, and is expressed in terms of per cent phosphorus of the total phosphorus to eliminate the need for measuring the microvolumes precisely. The elution step is always started with dilute ammonia because the phosphomolybdate complex is poorly soluble in acid. The total phosphorus content of the sample is determined on a separate aliquot colorimetrically after complete hydrolysis to orthophosphate.

Table V. Analysis of Very Dilute Phosphate Solutions

Sample	Phosphate Species	Concd. Solution (0.15% Phosphorus), % Phosphorus	Dilute Solution (<10 P.P.M. Phosphorus)		
			% Phosphorus Found	P.P.M. Phosphorus	
				Calcd. from concd. soln.	Found
Dilution in distilled water	Orthophosphate	40.2	41.6 41.6	3.09	3.20 3.20
	Pyrophosphate	34.6	34.5 34.4	2.66	2.66 2.65
	Triphosphate	25.5	23.9 24.1	1.96	1.84 1.86
Dilution in tap water, 75 to 100 p.p.m. hardness	Orthophosphate	40.2	40.8 41.1 39.8	3.09	3.14 3.16 3.06
	Pyrophosphate	34.6	33.8 33.9 35.5	2.66	2.60 2.61 2.73
	Triphosphate	25.5	25.5 25.1 24.7	1.96	1.96 1.93 1.90
Dilution in surface water, 293 p.p.m. hardness	Orthophosphate	40.2	41.8 41.0	1.21	1.25 1.23
	Pyrophosphate	34.6	36.6 36.2	1.04	1.10 1.09
	Triphosphate	25.5	21.6 22.8	0.77	0.65 0.68

A separate eluting step can be omitted, and the filter paper carried along instead in the phosphate solution if the recommended colorimetric phosphorus determination (*12*) is employed, and exactly the same conditions are maintained for all the fractions of one analysis. The filter paper does not interfere with the hydrolysis step. However, in the extraction of the phosphomolybdate complex by the organic solvent, as provided in the special colorimetric phosphate determination, the distribution of the phosphomolybdate complex between aqueous and nonaqueous phases seems to be replaced by a distribution among three phases: cellulose, aqueous phase, and organic solvent mixture. The effect is a deficiency of phosphorus in the organic solvent phase, which amounts to a definite percentage of the phosphorus for every unit of cellulose surface present. These complications are circumvented in routine work by attributing equal paper areas to all phosphate zones, carrying along all of the fractions of one analysis in a group to provide equal degrees of maceration of the paper, and determining only the ratios of each fraction to the total. Microgram quantities of phosphorus in each fraction cannot be measured as accurately when the paper is present during the extraction as they can be in the procedure which includes a separate elution of the phosphate from the paper prior to the extraction of the phosphomolybdate complex.

Satisfactory separation of the chain phosphates can be obtained only in acid chromatographic solvent mixtures. Partial hydrolysis of the condensed phosphates occurs, therefore, during a chromatographic separation; the resulting hydrolysis products are located on the chromatographic sheet according to the respective R_f values and the time which elapsed since their formation. Because triphosphate in solution degrades to 1 mole of pyro- and 1 mole of orthophosphate, less than one third of the phosphorus due to hydrolysis of triphosphate will be located above the pyro zone in an ascending run. By keeping the duration of the run as short as possible, by working at room temperature, and by applying corrections, the errors from hydrolysis can be minimized. The correction recommended earlier for acidic one-directional runs is based on three series of chromatographic runs which were carried out under exactly the same conditions over varying lengths of time ($2^1/_2$ to 10 hours). The data are shown in Figure 4. A corresponding increase was found for the percentage of phosphorus as pyrophosphate in runs of 5 hours or shorter duration. This indicates that, with the cutting procedure recommended, the major amount of the hydrolysis products is recovered in the pyro area in short runs, and that correction for the pyro- and triphosphate region only is adequate. The hydrolytic degradation of pyrophosphate proceeds considerably more slowly than that of the other phosphates; under the same acidic conditions pyrophosphate is about nine times more stable than is triphosphate. Quantitative evaluation of chromatograms showed about the same hydrolysis rates for pyro- and triphosphate as are known to take place in aqueous solution. Hydrolytic degradation of triphosphate in chromatographic runs is shown in Figure 4. The cellulose, therefore, does not seem to increase the hydrolytic degradation noticeably, and corrections for hydrolysis in paper chromatographic separations can be calculated from the published data valid for dilute aqueous solutions (*3, 7, 16, 19*). The ring phosphates undergo relatively fast hydrolysis in basic as well as in acidic solution, so that corrections for this effect should be made in highly precise determinations. Within the absolute accuracy given by the quantitative technique described herein, it is probably sufficient to use an estimated correction about the same as applied for hydrolysis of triphosphate.

The procedures recommended here keep the separating runs in the acid solvent as short as possible. It was found that after $2^1/_2$ hours at 25° C., complete separation of ortho-, pyro-, tri-, and long-chain phosphates is accomplished (Figure 5). The respective zones can easily be cut and the correction values for pyro- and triphosphate are relatively small (<1%), so that a slight deviation in room temperature during the run does not cause an appreciable error in the correction.

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RECEIVED for review December 22, 1955. Accepted March 30, 1956. Division of Analytical Chemistry, 129th Meeting, ACS, Dallas, Tex., April 1956.

Color Reaction of Hexuronic Acids with Anthrone

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The influence of various experimental factors on the color reaction of glucuronic and galacturonic acids with anthrone in 27.5*N* sulfuric acid has been investigated. While hexoses, methylpentoses, and pentoses react with anthrone to give a blue-green color with maximum absorbance at 620 to 625 mμ, uronic acids produce a pink to red color with maximum absorbance at 540 to 550 mμ. The anthrone–uronic acid color does not immediately begin to diminish in intensity as is the case with other carbohydrates; it increases in intensity with age, reaching a maximum after about 44 hours. Under suitable conditions the magnitude of anthrone–uronic acid absorbance at 540 to 550 mμ is of the same order as that reported in the literature for pentoses at 620 to 625 mμ. Mixtures of glucuronic acid with glucose and galacturonic acid with galactose obey Beer's law and are additive in all proportions.

IN THE literature pertaining to the anthrone method of carbohydrate analysis there are only occasional references to the estimation of uronic acids (*2, 3, 6*). Moreover, these compounds have invariably been examined under experimental conditions optimum for some other class of carbohydrates. The present report, covering the behavior of hexuronic acids with anthrone in strong sulfuric acid, is aimed at filling this hiatus.

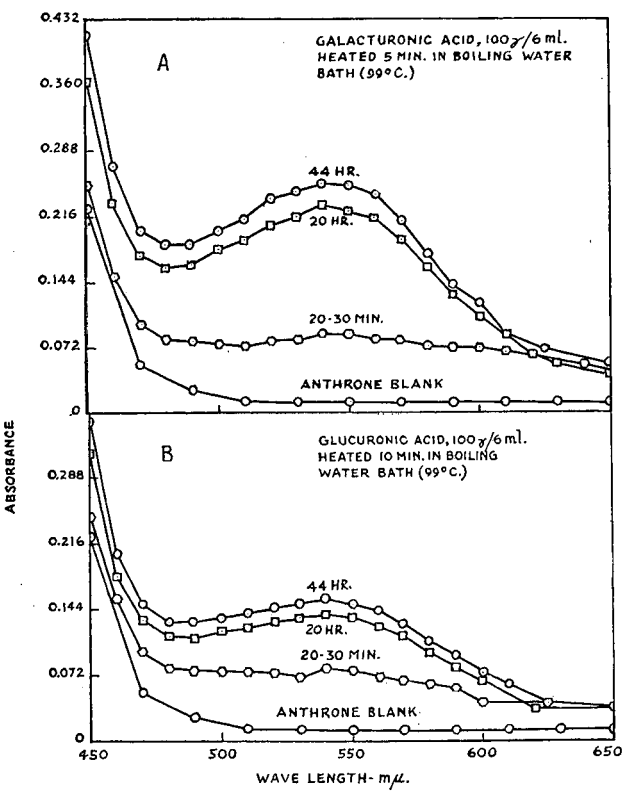

Figure 1. Absorbance spectra

A. Anthrone–galacturonic acid
B. Anthrone–glucuronic acid

PROCEDURE

The materials and apparatus employed in this investigation were the same as those described (*3*), with the following exceptions: Anthrone from the Nutritional Bio-chemicals Corp. was used exclusively, and some of the photometric readings were made on the Spectronic 20 (Bausch & Lomb) as well as the Beckman DU photometer.

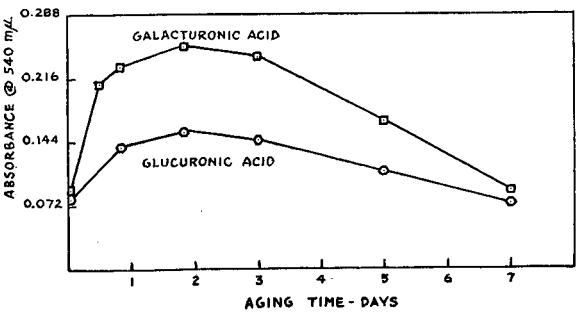

Figure 2. Influence of age upon developed anthrone–uronic acid color

Glucuronic acid heated 10 minutes in boiling water bath (99°)
Galacturonic acid heated 5 minutes in boiling water bath (99°)
Concentration. 100 γ per 6 ml.

Anthrone solutions are prepared by dissolving 0.160 gram of this reagent in 100 ml. of 27.5 ± 0.1*N* sulfuric acid, allowing about 60 minutes for complete solution. Uronic acid solutions are also prepared by dissolving solid sample in 27.5*N* sulfuric acid. In this case about 30 minutes is allowed to effect complete solution.

The basic procedure employed for most of the determinations in this work is as follows. Four milliliters of anthrone solution are pipetted into uniform borosilicate glass test tubes, followed by 2 ml. of uronic acid solution. As both reactants are dissolved in 27.5*N* sulfuric acid, no heat of mixing is evolved. Test samples are then heated in a boiling-water bath or, if lower temperatures are desired, in a thermostatically controlled water bath. After heating, samples are immediately transferred to a cold-water bath (4° ± 1° C.) for 3 minutes. Heating and cooling time is measured to ±2 seconds, and the test tubes containing the reacting solutions are spaced in wire racks in the heating and cooling baths to facilitate uniform heat transfer. After removal from the cold-water bath, test samples are held at room temperature (23° ± 2° C.) in a lightproof cabinet, usually for 20 hours. Photometric readings are taken at 540 to 550 mμ.

EXPERIMENTAL RESULTS AND DISCUSSION

Selection of Wave Length. Most carbohydrates react with anthrone, under the conditions employed here, to give a blue-green color with maximum absorbance at 620 to 625 mμ. The uronic acids react to give a red color with maximum absorbance at 540 to 550 mμ (Figure 1, *A* and *B*).

A similar type of behavior has been reported for several other carbohydrates. Sattler and Zerban (*5*) observed that L-ascorbic acid and anthrone react to produce a cherry-red color. More recently, Koehler (*4*) has reported that 2-deoxy sugars react with anthrone to yield colors with absorption maxima ranging from 520 to 560 mμ.

Stability of Developed Color. Figure 1 delineates another aspect of uronic acid behavior, which diverges from the usual pattern: The intensity of the developed color does not promptly begin to diminish, but rather increases. When photometric read-

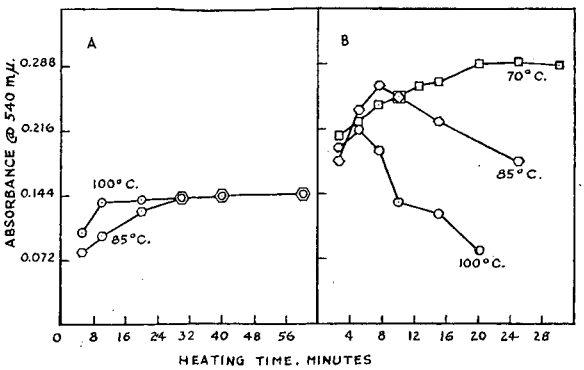

Figure 3. Influence of heating time on absorbance at various temperatures

A. Glucuronic acid, 100 γ per 6 ml., aged 20 hours
B. Galacturonic acid, 100 γ per 6 ml., aged 20 hours

ings are taken 20 to 30 minutes after removal from the cold-water bath, color development is minimal and there is only an incipient maximum. At this time the characteristic pink to red coloration is not yet visible. After 20 and 44 hours, however, color intensity has increased markedly and a definite maximum has developed.

The intensity of the anthrone–glucuronic acid color aged 20 hours is about double that aged 30 minutes; in the same age interval the anthrone–galacturonic acid color has almost tripled. Although equal concentrations of both uronic acids produce about the same amount of color when read at 20 to 30 minutes, galacturonic acid produces about 50% more color than glucuronic when both are read at 20 hours. At the latter time the magnitude of anthrone–uronic acid absorbance at 540 mμ is of the same order as that reported elsewhere (*3*) for pentoses at 625 mμ. Although maximum color development is reached at about 44 hours (Figure 2), the difference in absorbance at 20 and 44 hours is relatively small; the shorter, more convenient time interval has been arbitrarily selected as standard.

Time-Temperature-Absorbance Data. The influence of temperature on the rate and extent of color formation is summarized in Figure 3. The behavior of glucuronic acid is reminiscent of that of the ketohexoses at 60° C. (*3*). The use of temperatures below 100° C. serves only to increase the heating time required to attain maximum color development. Galacturonic acid, on the other hand, is more typical, in that heating beyond

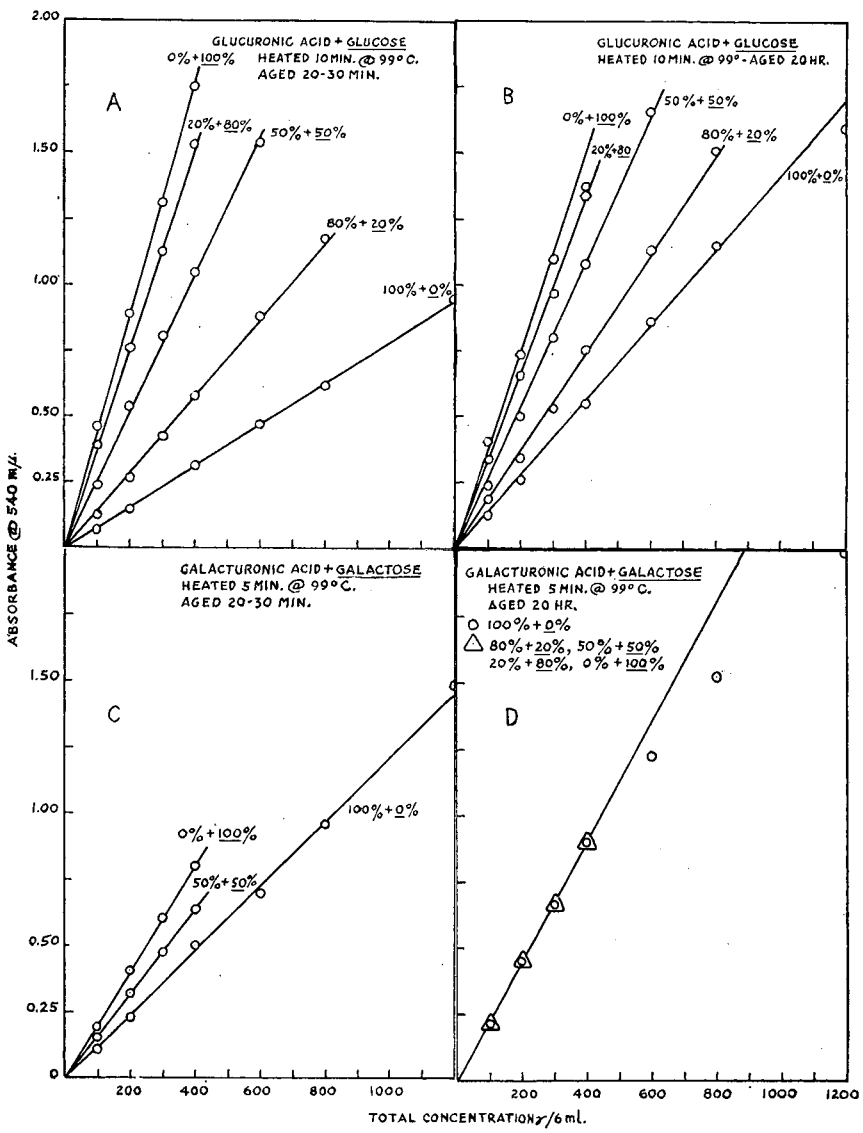

Figure 4. Influence of concentration on absorbance for uronic acid–aldohexose mixtures

the time at which maximum color development is attained results in color loss. However, as the temperature is lowered, the absorbance increases. At temperatures much below 70° C. the heating times required to attain maximum color become excessive.

Beer's Law and Additivity. Conformity to Beer's law has been checked for both glucuronic and galacturonic acids as well as mixtures of each uronic acid with its corresponding aldohexose. The results are presented graphically in Figure 4. The circles, etc., represent empirical points, while the solid lines are the least-mean-square straight lines through these points and the origin.

Color intensity at 20 hours is less dispersed for the various mixtures than at 20 to 30 minutes. This circumstance arises from the fact that the uronic acid color increases with age, while the aldohexose color decreases. In the case of galacturonic acid and galactose, this process results in both pure components having the same absorbance index, up to the point where Beer's law fails. Furthermore, Beer's law fails with galacturonic acid at appreciably lower concentrations than is the case with glucuronic acid.

The 80% + 20% and 20% + 80% mixtures were omitted in Figure 4, *C*, for the sake of clarity. However, both mixtures obey Beer's law as well as those shown, up to concentrations of 400 and 600 γ per 6 ml., respectively.

The component colors of the mixtures considered in Figure 4 are additive for total concentrations up to at least 400 γ per 6 ml. Deviations of observed from calculated values are all within the limits of experimental error. These same mixtures were also examined at a wave length of 620 mμ; they likewise obey Beer's law and are additive in all proportions at this wave length.

Influence of Reagent Age. Previous experiments (*3*) have indicated that reagent solutions analogous to those employed in this work are apt to be time-variable. Therefore, the influence of the age of such solutions upon the intensity of the anthrone–uronic acid color was examined.

Solutions of anthrone, glucuronic acid, and galacturonic acid were prepared in 27.5*N* sulfuric acid 1 hour and 24 hours prior to use. Both anthrone solutions were used with both solutions of each uronic acid. Anthrone–glucuronic acid color was developed by heating for 10 minutes at 100° C., while anthrone–galacturonic acid color was developed by heating for 25 minutes at 70° C.; in both cases full color development was attained during a holding period of 20 hours subsequent to heating. The statistical significance or nonsignificance of the influence of reagent age, as reflected in the absorbances of the four possible combinations of anthrone with each uronic acid, was determined from an analysis of variance. Further, by replicating each anthrone–uronic acid combination it was possible to ascertain the influence of reagent age upon the precision.

From the foregoing experiments the following conclusions were reached (at 0.99 probability level). The age of anthrone solutions, up to 24 hours, has no significant influence on color intensity with either glucuronic or galacturonic acid. The age of glucuronic acid solutions, up to 24 hours, also has no significant influence on color intensity. Galacturonic acid solutions, however, aged for a like period produce a significantly higher level of color. Finally, the color intensity obtained with either fresh or aged reagent solutions is equally reproducible.

Influence of Acid Concentration. Solutions of anthrone and the uronic acids were prepared in 26.5, 27.5, and 28.5*N* sulfuric acid, adjusted to within $\pm 0.1N$ of the indicated value. Test solutions of glucuronic acid were heated 10 minutes at 100° C., while those of galacturonic acid were heated 25 minutes at 70° C. The uronic acid concentration employed in both cases was 100 γ per 6 ml. of reaction mixture.

The three different concentrations of solvent acid caused very significant differences in the level of color intensity produced by each uronic acid. Care must therefore be exercised in the preparation of the solvent acid; a tolerance of $\pm 0.1N$ has been found satisfactory. The color intensity produced by both uronic acids increases with the solvent acid concentration. At 28.5*N* the level of color is about equal to that obtained with Dische's carbazole method (*1*).

Table I. Effect of Procedural Variations

Procedural Variation	Absorbance[a] Glucuronic acid	Galacturonic acid
A	0.126	0.193
B	0.143	0.161
C	0.158	0.230

[a] Each entry is the mean of five separate determinations. Concentration of uronic acid in every case was 100 γ per 6 ml.

Table II. Precision of Determinations

	Heating Conditions Min.	° C.	No. of Runs	Total No. of Detns.	Std. Dev., $\hat{\sigma}$
Glucuronic acid	10	100	8	34	0.004
Galacturonic acid	25	70	7	28	0.002

In all cases photometric readings were taken 20 hours subsequent to heating. Absorbance values were mainly in the 0.200 to 0.300 range.

Comparison of Variations in Procedure. Three procedural variations were considered.

A. Uronic acid in 27.5*N* sulfuric acid is added to anthrone in the same solvent, mixed at room temperature, heated, etc. (this is the basic procedure employed throughout this work).
B. Uronic acid in 27.5*N* sulfuric acid is first heated alone, then anthrone in the same solvent is added, both solutions at room temperature.
C. Uronic acid in water (1 part by volume) is layered onto a chilled solution of anthrone in concentrated sulfuric acid (2 parts by volume), mixed, heated, etc. In the last instance a cooling mixture of crushed ice and salt is employed, in order to keep the temperature below 70° C. when the layered solutions are mixed.

An analysis of the data summarized in Table I indicates that Procedure B produces a higher level of color than Procedure A with glucuronic acid, but a lower level with galacturonic acid. Procedure C produces a higher level of color than either Procedure A or B with both uronic acids. This latter observation is principally accounted for by the fact that 1 volume of water plus 2 volumes of the concentrated sulfuric acid used produces a normality somewhat higher than 27.5.

All three procedures are capable of approximately the same precision, although Procedure C tends to be less precise than the other two. The precision obtained with Procedure A, when photometric readings are taken at 550 mμ, is summarized in Table II. The pooled estimate of the standard deviation, $\hat{\sigma}$, was calculated as previously (*3*).

ACKNOWLEDGMENT

The authors are indebted to L. C. Massopust and F. W. Faust for the preparation of drawings and photographs and to Sister Mary Pauline-Joseph for assistance in some of the experimental work. This work was supported by a grant-in-aid from Lakeside Laboratories, Inc., Milwaukee, Wis.

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RECEIVED for review January 13, 1956. Accepted April 11, 1956. Presented in part before Division of Carbohydrate Chemistry, 128th Meeting, ACS, Minneapolis, Minn., September 1955.

Electrolytic Separation and Volumetric, Absorptiometric, and Coulometric Estimation of Thallium

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Thallium may be quantitatively separated on the anode from an ammoniacal solution containing silver ion as a cathodic depolarizer. The mean of 41 determinations gives 116.4 as equivalent weight of the oxide, with a standard deviation of 0.35. Under certain conditions the deposit of silver on the cathode is a precise measure of the thallium. Thallium(III) may be precisely estimated by back-titrating the solution of thallium at pH 3.5 with thorium nitrate and excess EDTA. Thallium(I) may be measured by a spectrophotometric titration at 222 mμ with standard EDTA. Thallium(III) may be estimated absorptiometrically from measurements on the chloroform extracts of thallium dibenzyldithiocarbamate at 430 mμ. Thallium(I) may be estimated with an accuracy within 0.3% by direct coulometric oxidation at the anode in an acid solution.

EXPERIMENTS were carried out to determine the best conditions for the electrogravimetric separation of thallium from solution and for its volumetric, absorptiometric, and coulometric estimation.

ELECTROGRAVIMETRIC SEPARATION OF THALLIUM

This is a report of a study of the controlled potential deposition of thallium oxide at the anode.

Heiberg (*6*) deposited thallium on the anode as a higher oxide by electrolyzing a thallous sulfate solution in the presence of acetone at 50° C. He considered the deposit to be Tl_2O_3. In three determinations he found 81.22, 81.29, and 81.14% thallium in thallous sulfate, the theoretical content of which is 80.97% thallium. Gallo and Cenni (*3*) deposited thallium oxide at the anode from an oxalic acid solution of thallous sulfate and concluded that the deposit was Tl_3O_5. Tzentnershver and Trebackziewicz (*16*) asserted that the deposit obtained on the anode was TlO_2. Gutbier and Dieterle (*4*) found that the compound formed at the anode was Tl_2O_3 contaminated by as much as 1% sulfate. Besson (*1*) showed that the deposit formed on the anode was Tl_2O_3 more or less hydrated. He claimed that it was difficult to deposit thallium oxide quantitatively on the anode because of its tendency to deposit simultaneously on the cathode. Peltier and Duval (*12*) showed that thallic oxide prepared at the anode retains water very tenaciously. There is an almost horizontal region between 156° and 283° C. on the graph from the thermobalance corresponding to Tl_2O_3, and there is another flat region between 411° and 677° C. corresponding to 3 $Tl_2O_3 . Tl_2O$. Norwitz (*11*) deposited thallium on the anode as the oxide from an ammoniacal ammonium nitrate solution containing copper ions, which act as a depolarizer and prevent the deposition of metallic thallium on the cathode. Some copper is deposited with thallic oxide and a correction must be made for this element.

In this study of the quantitative removal of thallium from solution as an oxide, a potentiostat was used. The equivalent weight of the anode deposit was determined by weighing the amount of silver simultaneously deposited and finding the weight of thallium oxide corresponding to one atomic weight of silver.

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Apparatus. A potentiostat similar in all respects to that described by Lamphere (*7*) and a saturated calomel electrode with an agar-agar salt bridge were used. The electrolysis cell was a 400-ml. beaker and the electrolyte was stirred by a polyethylene-covered stirring bar driven by a magnetic stirrer. The electrodes were platinum gauze about 5 cm. high and 2.5 and 1.5 cm. in diameter. In the silver coulometer high purity silver foil was used as anode and a bright platinum foil was used as cathode. When a large amount of electricity was measured, a spiral silver cathode was used. The external silver coulometer cell was prepared by joining two 150-ml. beakers together with 10-mm. tubing having a fritted-glass disk of medium porosity. This arrangement prevented errors from anode slime.

Reagents. Two samples of thallium sulfate were used. One lot, from A. D. Mackay, New York, N. Y., assayed 99.9% by the chromate method. Another lot was prepared from a sample of Kahlbaum metal, which was dissolved in nitric acid, converted to the sulfate, and recrystallized repeatedly. The sulfate was converted to the chloride, which was reconverted to the sulfate after rejection of the mother liquor. The sulfate was electrolyzed using platinum electrodes. The anode was separated from the cathode by an inverted U-tube. The metallic crystals were removed from the cathode from time to time and washed. The crystals were dissolved in nitric acid and the nitrate was converted to the sulfate. Some of the Mackay salt was similarly treated.

Ammonium hydroxide, ammonium nitrate, and silver nitrate were of analytical reagent grade.

The electrolyte consisted of 250 ml. of a solution of silver nitrate and thallium nitrate or sulfate buffered to a pH of 9.5 with 4 grams of ammonium nitrate and 8 ml. of ammonium hydroxide. The anode potential was kept at +0.70 volt with reference to the normal hydrogen electrode. The controlled potential apparatus made the operation automatic and did not permit the evolution of oxygen.

Table I shows the results of a typical run. In this instance the potentiostat was not preset to the equilibrium voltage, so that the instrument took several minutes to reach equilibrium. The experiment was repeated by dissolving the thallium oxide and replating it. On the second trial the silver weighed 0.1031 gram and the oxide weighed 0.1111 gram.

The data of Table I contain information of interest. The original sample of thallium sulfate weighed 0.1203 gram, which should yield 0.1089 gram of thallium oxide instead of the measured value of 0.1109 gram. In most of the runs the anode deposit was tested for the presence of silver; in no instance was the test positive. The procedure used to dry the deposit was modified in many ways, but the results were always the same. After the deposit was washed with water, it was dried at 110° C.;

Table I. Controlled Potential Analysis of Thallium(I) Sulfate

Tl_2SO_4, gram	0.1203
$AgNO_3$, gram	0.2
Oxide, gram	0.1109
Silver metal, gram	0.1031

Volume, 300 ml. pH 9.5. Temp., 24° C.

Time	Volts	Ampere
10.51	2.9	0.0008
10.52	3.5	0.004
10.54	4.8	0.080
10.55	5.6	0.150
10.57	6.6	0.175 (equilibrium)
11.00	5.8	0.120
11.05	5.0	0.050
11.10	4.8	0.015
11.20	4.0	0.004
11.40	2.0	0.0002

Table II. Electrogravimetric Estimation of Thallium

Sample	Sample Size, Gram	Silver, Gram	Oxide, Gram	Sample, Gram, Based on Silver, Eq. Wt. 116.4	
TlCl	0.2084	0.1875	0.2023	0.2084	0.2085
	0.1816	0.1631	0.1753	0.1814	0.1809
Tl_2SO_4	0.3380	0.2885	0.3107	0.3383	0.3372
	0.9539	0.8147	0.8804	0.9530	0.9545
	0.5768	0.4916	0.5312	0.5764	0.5760

the same results were achieved if the deposit was dried at 230° C. If the platinum gauze with deposit was placed in a small beaker and left at 230° C. in the oven overnight, there was no loss in weight. At times some of the deposit fell off the gauze into the beaker, but all the material was accounted for to 0.1 mg. This is not in harmony with the findings of Peltier and Duval (*12*).

If the anode deposit is Tl_2O_3, the gram equivalent weight should be 114.2. In all determinations the number of equivalents of thallium oxide based on this factor was greater than the number of equivalents of silver deposited on the cathode. The jet-black deposit was very compact and adherent and it seemed unlikely that it contained absorbed salt. The most convenient estimate of the true equivalent weight of the anode deposit should be obtained by using the silver on the cathode as an internal coulometer. There was a risk, however, that some impurity or some unusual condition would invalidate the determination. For example, if the solution contained thallic ion, a correspondingly smaller amount of silver would be deposited.

To minimize errors from impurities, the following procedure was adopted.

To 400 ml. of water were added 4 grams of ammonium nitrate, 8 ml. of concentrated ammonium hydroxide, 0.4 to 4 grams of thallium sulfate, and 0.5 to 5 grams of silver nitrate. In some of the dilute solutions thallium chloride rather than thallium sulfate was used. The weights of thallium and silver salts chosen were roughly four times those needed to supply metal for the expected electrode deposits. The solution was electrolyzed with a current of 40 ma. without the use of the controlled potential apparatus. After about 30 mg. of silver was deposited, the electrodes were removed, freed from their deposits, dried, and weighed. The electrolysis was resumed with a current of 40 ma. When the desired weight of silver had been deposited (estimated from the current and the time) and while the solution still contained liberal amounts of thallium and silver ions, the electrodes were removed, washed with water, dried at 170° C., cooled, and weighed. From the weight of the silver deposited on the cathode and the weight of oxide on the anode the equivalent weight of the anode deposit was calculated. The mean of 41 determinations was 116.4, with a standard deviation of 0.35. The values ranged from 115.6 to 117.1 and the weight of oxide ranged from 0.05 to 1.2 grams.

Gallo and Cenni (*3*) obtained results in which the weight of oxide was higher than it should be if the formula were Tl_3O_5 (here the equivalent weight is 115.5). They dried their deposit at 160° C. while passing over it a stream of dry, carbon dioxide–free air; the weight and analysis corresponded to Tl_3O_5. They implied that when the anode deposits were dried in air there was a reaction with carbon dioxide. In this work some of the deposits were placed in a vacuum oven which was heated after it had been evacuated. After the oven had cooled, the electrode with deposit was weighed, placed in an ordinary drying oven, and heated to 160°. No gain in weight was noted, and the equivalent weight averaged 116.1. This indicates that the oxide does not combine with carbon dioxide at the drying temperature.

An effort was made to measure the current density by means of an external coulometer, using a 15% solution of silver nitrate. One electrode was sheet silver, while the other was a platinum foil to which was spot-welded a platinum lead. The anode and cathode compartments were separated by a fritted-glass plug. The weight of silver deposited on the cathode was the same as the weight of silver deposited on the cathode of the silver coulometer.

In another experiment two electrolysis cells were connected in series. The weight of silver was 0.2276 gram on each cathode, while the anode deposits weighed 0.2448 and 0.2452 gram. A similar experiment yielded 0.1883 and 0.1882 gram for the cathode deposits, while the anode deposits were 0.2037 and 0.2032 gram.

Some further experiments were performed in which the number of coulombs passing through the electrolysis cell was calculated by measuring the area under the time-current curve. A precision potentiometer was used to measure the internal resistance drop through a standard resistor and the time was measured with a precision electric timer. In one determination the measured weight of silver was 1.1794 grams and the calculated weight was 1.1790 grams. The use of this electrolyte, ammoniacal silver nitrate and thallium sulfate, in a simple coulometer will be reported in another paper.

Table II gives results obtained in the analysis of thallium salts by electrolysis. Thallium may be quantitatively separated at the anode by controlled potential electrolysis. The weight of the oxide deposit is greater by almost 2% than the theoretical weight based on pure Tl_2O_3. This may be due to retained water. It has been observed that the composition of the oxide is not definite. If interfering ions are absent, so that the anode and cathode reactions involve only thallium and silver, respectively, the weight of silver should be a more precise measure of the thallium—that is, the number of equivalents of silver times one half the atomic weight of thallium should give the weight of thallium in the anode deposit. If interfering ions are present in the plating of the anode deposit, the deposit may be dissolved in sulfurous acid and converted to the neutral sulfate. The thallium oxide may now be plated with the aid of a potentiostat, so that the anode and cathode reactions involve only thallium and silver. One of the following methods may also be used for estimation of thallium.

VOLUMETRIC ESTIMATION OF THALLIUM(III) WITH EDTA

The use of EDTA, (ethylenedinitrilo)tetraacetic acid, to estimate thallium has been reported by Pribil (*13*) and Flaschka (*2*), who used a replacement titration of pH 10. In this work thallium(III) has been determined at pH 3.5 by using excess (ethylenedinitrilo)tetraacetic acid and then titrating the excess with standard thorium nitrate and alizarin S. It was found that the presence of thallium(I) does not interfere with this titration, as it does with the back-titration at pH 10. This is to be expected, because Pribil and Zabronsky (*14*) with the aid of a polarograph showed that the half-wave potential of thallium(I) is not influenced by (ethylenedinitrilo)tetraacetic acid in an acid medium but is shifted in an alkaline medium.

Reagents. (Ethylenedinitrilo)tetraacetic acid disodium salt, 0.05*F*. Dissolve 18.6 grams of the salt in 1 liter of solution.

Thorium nitrate, 0.05*F*. Dissolve 27.6 grams of thorium nitrate tetrahydrate in 1 liter of solution.

Standard zinc nitrate, 0.05*F*. Dissolve 3.27 grams of highest purity zinc (accurately weighed) in a little nitric acid. Dilute to the mark at 20° C. in a 1-liter volumetric flask.

Chloroacetate buffer. Dissolve 13.6 grams of sodium acetate trihydrate, 1*F*, in 1 liter of water. Dissolve 189 grams of monochloroacetic acid, 2*F*, in 1 liter of water.

Buffer, pH 10. Add 60 grams of ammonium chloride to 570 ml. of ammonium hydroxide and dilute to 1 liter.

Alizarin S indicator. Dissolve 0.1 gram of alizarin S in 100 ml. of water.

Eriochrome Black T indicator. Dissolve 0.1 gram of Eriochrome Black T in 100 ml. of water.

Standardization of EDTA [(Ethylenedinitrilo)tetraacetic Acid]. Place an aliquot of the 0.05*F* zinc nitrate solution in a 500-ml. Erlenmeyer flask and dilute to 250 ml. Add 15 ml. of the buffer solution (pH 10), followed by 2 ml. of the Eriochrome Black T indicator. Add the 0.05*F* EDTA to the zinc solution from a buret until a sharp change in color from red to blue is observed.

Standardization of Thorium Nitrate. To an aliquot of standard EDTA add 2 ml. of alizarin S indicator, 5 ml. of monochloro-

acetic acid, 8 ml. of sodium acetate, and enough water to bring the volume to about 100 ml. The pH should be 2.8. Add the thorium nitrate from a buret until a sharp change in color from yellow to orange-red occurs.

Titration of Thallium at pH 3.5. To a solution containing 50 to 200 mg. of thallium(III) in a volume of 100 ml. add an excess of standard EDTA. Buffer the solution carefully to pH 3.5 by adding 4 ml. of monochloroacetic acid and the required amount (usually 8 to 14 ml.) of sodium acetate. Titrate the excess EDTA with standard thorium nitrate in the presence of 2 ml. of alizarin S indicator. Table III gives some results obtained by this method.

The mean of 14 determinations with EDTA and thorium nitrate yielded 200.8 mg. of thallium for a 20-ml. aliquot of the thallic solution at pH 3.5. Eight determinations with the EDTA zinc nitrate method at pH 10 also gave an average value of 200.8 mg. This value was further checked electrogravimetrically, using the ammoniacal silver nitrate depolarizer and a controlled anode potential. Based on the equivalents of silver deposited, the value of 200.8 mg. of thallium was obtained.

The interferences mentioned by ter Haar and Bazen (*5*) would also interfere with the thorium nitrate titration for thallium. These include copper, lead, zinc, cadmium, aluminum, iron, nickel, cobalt, bismuth and manganese cations and fluoride, phosphate, and oxalate anions.

ABSORPTIOMETRIC ESTIMATION OF THALLIUM(III)

With diethyldithiocarbamate many of the heavy metals give slightly soluble products, most of which are soluble in carbon tetrachloride or chloroform. A few of these, including thallium, are strongly colored. The thallium complex is so sensitive to light that it cannot be used for colorimetric purposes. Martens and Githens (*10*) have described a method for determining copper using zinc dibenzyldithiocarbamate instead of sodium diethyldithiocarbamate. The resulting colored complex is much more stable toward light than the complex formed from the diethyldithiocarbamate. In this work it has been found that the thallium complex of dibenzyldithiocarbamate is stable in subdued light and provides a means of moderate sensitivity for the estimation of thallium(III).

Table III. Titration of Thallium(III) at pH 3.5

Tl Taken, Mg.	Tl Found, Mg.	Δ, Mg.
50.21	49.92	0.29
	50.15	0.06
	50.64	0.43
Mean	50.23	0.02
100.4	100.1	0.3
	100.8	0.4
Mean	100.45	0.05
150.6	149.6	1.0
	151.7	1.1
Mean	150.65	0.05
200.8	200.2	0.6
	201.0	0.2
	201.1	0.3
Mean	200.7	0.1

Reagents. Zinc dibenzyldithiocarbamate. Purify a sample of the solid (sold by Naugatuck Chemicals, Elmira, Ont., as a rubber accelerator under the trade name of Arazate) by recrystallizing from benzene and dissolve 3 grams in a liter of chloroform.

Thallium(III) sulfate. Weigh a 1.26-gram sample of thallium(I) sulfate and dissolve it in a small volume of 1*N* sulfuric acid. Acidify with 5 ml. of sulfuric acid and oxidize with chlorine gas. Boil to expel chlorine and then make up to 500 ml. in a volumetric flask. From this stock solution prepare a solution containing 400 mg. of thallium per liter.

Apparatus. A Beckman DU spectrophotometer with 1-cm. Corex cells is used.

Procedure. Prepare a stock solution of the thallium complex by extracting an aqueous solution containing 50 mg. of thallium(III) with a chloroform solution containing 450 mg. of Arazate. Dilute the separated chloroform layer to 250 ml. From this stock solution prepare solutions containing 0.5, 1.0, 2.5, 5.0, 7.5, and 10 mg. of thallium per 50 ml. of chloroform. Measure the absorbance of these solutions at 430 mμ. The molar absorbance index of the complex at 430 mμ in chloroform at 25° C. is 1340.

Because this complex obeys Beer's law, the method may be used as a moderately sensitive one for the estimation of thallium(III). The interfering elements mentioned by Martens and Githens (*10*) for copper apply here. These include antimony, bismuth, cobalt, mercury, nickel, and silver.

SPECTROPHOTOMETRIC TITRATION OF THALLIUM(I) WITH EDTA

The spectrophotometric titration of thallium(I) with EDTA was studied in an alkaline medium. The titration was made possible by the relatively high absorbance of the chelate in the ultraviolet region. Evidence of a complex between EDTA and thallium(I) was obtained from the volumetric determination of thallium(III), where the presence of the unipositive thallium interfered with the estimation of thallium(III) at pH 10. A study of the wave-length–transmittancy curves for thallium chloride, the thallium complex of EDTA, and finally EDTA, all at pH 10, showed that 222 mμ was a suitable wave length.

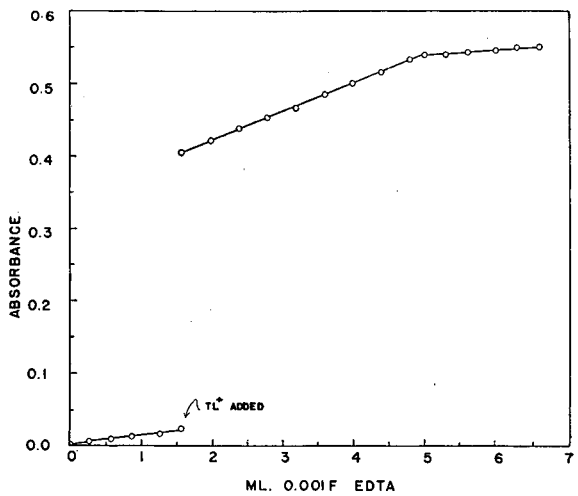

Figure 1. Spectrophotometric titration of 5.00 ml. of 0.001*F* thallium(I) chloride with 0.001*F* EDTA

Apparatus. The ultraviolet attachment for the Beckman spectrophotometer is used for these measurements.

Reagents. Prepare a 0.001*F* thallium(I) solution by dissolving 0.1199 gram of the chloride in water and diluting to 500 ml. Prepare 0.001*F* solution of EDTA by dissolving 0.1862 gram of the salt in enough water to give 500 ml. of solution. Prepare the ammonium hydroxide–ammonium chloride buffer by adding 30 grams of ammonium chloride to 280 ml. of ammonium hydroxide and diluting to 500 ml.

Procedure. Add 2 ml. of buffer to 100 ml. of water in a 250-ml. beaker. Add the EDTA solution to this in measured portions from a microburet and measure absorbance at 222 mμ after each addition of reagent. After the addition of between 1 and 2 ml. determine whether the plot of volume against absorbance has a constant slope. If the slope is constant, no impurities are present. If there is an abrupt change in slope, the reagent volume required to neutralize the impurity is that which was needed to bring about the change in slope. Now pipet into the beaker 5.0 ml. of the 0.001*F* thallium(I) chloride and continue the titration. The results for a typical titration are shown by Figure 1.

The results of the titration of thallium(I) with EDTA show that a chelate was formed. In all instances a definite spectro-

photometric end point is reached and the reproducibility indicates that the method could be used for the spectrophotometric titration of thallium(I) by the technique adopted by Sweetser and Bricker (*15*) for the titration of magnesium, calcium, etc.

COULOMETRIC OXIDATION OF THALLIUM

Between 0.3 and 1.0 meq. of thallium may be estimated by primary coulometric analysis at a platinum anode in an acid solution with an accuracy within 0.3%. The procedure and apparatus are the same as those employed by MacNevin and Baker (*9*), except that the electrolyte (1*M* sulfuric acid) is scavenged at an anode potential of +1.38 volts *vs.* the S.C.E. for 15 minutes. The anode potential is reduced to +1.34 volts and the thallium(I) solution is added. The oxidation is considered to be over when the current falls to 0.2 ma.

The primary coulometric oxidation of thallium has more theoretical than practical interest. The anodic oxidation of thallium(I) did not obey the equation proposed by Lingane (*8*) for single reactions occurring with 100% efficiency:

$$i_t = i_0 e^{-kt} \quad (1)$$

where i_0 is the current at zero time, i_t is the current at time t, and k is given approximately by

$$k = \frac{DA}{Vd} \quad (2)$$

where D is the diffusion coefficient in square centimeters per second, d is the thickness of the diffusion layer, A is the electrode area, and V is the volume in milliliters. As the plot of log current against time did not give a straight line, the reaction is doubtless complex. MacNevin and Baker (*9*) report similar findings for the primary coulometric oxidation of arsenic(III).

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RECEIVED for review November 26, 1955. Accepted April 7, 1956. Work supported by the Defence Research Board of Canada Grant 7510-19, Project D 44-75-10-19.

Analysis of Cyclopentadiene Dimer Concentrates

Review of Existing Methods and Description of Mass Spectrometer Method

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Existing methods for cyclopentadiene analysis have been reviewed. A new analytical method utilizing the mass spectrometer is described in which cyclopentadiene dimer concentrates are depolymerized in a specially constructed cracking chamber attached to the inlet system of the mass spectrometer. The method is accurate, reproducible, rapid, and ideal for plant and product quality control in a cyclopentadiene recovery process.

IN A process for the recovery and purification of cyclopentadiene, complex mixtures of the dimers, codimers, and higher polymers of cyclopentadiene and its homologs and acyclic pentadienes, such as piperylene and isoprene, are encountered. Because cyclopentadiene itself is the component of most commercial importance, it was necessary to obtain an accurate, reproducible method for determining cyclopentadiene in the presence of the less valuable components. This paper contains a brief discussion of several analytical methods which had been used and a more detailed description of a mass spectrometer method which has been developed and is currently in use.

A true analysis of such dimer concentrates would show the total quantity of the various compounds that are actually present. However, the basic information desired is the quantity of the individual monomers, particularly cyclopentadiene, which could be recoverable by some depolymerization process. The degree of depolymerization of a dimer concentrate is dependent upon temperature, residence time, and pressure (vapor phase). It is also a function of the types of dimers present in the concentrate itself—for example, cyclopentadiene dimer is more easily cracked to monomer than is the codimer of cyclopentadiene and isoprene. Also, because it is difficult to crack cyclodiene dimers without some degradation, any method of analysis which is based on depolymerization can represent only the quantity of monomers available under the specific cracking conditions employed. Theoretically, at least, the cracking conditions that produce the greatest amount of depolymerization with the least amount of degradation should permit the most accurate determination of available cyclopentadiene. Incomplete or inconsistent depolymerization has been primarily responsible for the difficulties encountered with most of the analytical methods that have been tried.

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PREPARATION OF ANALYTICAL STANDARDS

Pure dimers of cylcopentadiene, methylcyclopentadiene, and dimethylcyclopentadiene were prepared in the laboratory by conventional methods (*2*, *6*). The final products were vacuum distilled to remove traces of low boiling and high boiling impurities. Mass spectrometer analyses of heart cuts from these distillations showed less than 1% of the adjacent homologs and, for calibration purposes, the dimers were considered to be essentially 100% pure. The crystalline dicyclopentadiene which was obtained compared favorably with the same material isolated by Edson, Powell, and Fisher (*2*).

Qualitative mass spectrometer analysis of the cracked products from plant dimer concentrates indicated the presence of significant concentrations of thermally stable codimers of cyclic pentadienes and acyclic pentadienes; therefore, it was essential to obtain these codimers for calibration purposes. Codimer concentrates of approximately 90% purity were isolated from the dimer concentrate by vacuum distillation and were also synthesized from pure isoprene, cyclopentadiene, and methylcyclopentadiene. Dipen-

tene, another known impurity, was obtained from Eastman Kodak Co.

AVAILABLE ANALYTICAL METHODS

The analytical methods considered may be classed into two types: those which determine cyclopentadiene and methylcyclopentadiene dimers directly and those which determine the cracked products of those dimers and various codimers. The first type was completely unsatisfactory, because of the complex nature of the product. The formic acid method of Bergman and Jophe (*1*) and an infrared method studied in this laboratory both fall in this category. In the second group are the fulvene method (*4, 5*), the exhaustive tube cracking method, the ultraviolet method (*3*), and the mass spectrometer method described here.

The fulvene method of Uhrig, Lynch, and Becker (*5*), which uses benzaldehyde to form the phenylfulvene, has been used successfully for the determination of low concentrations of cyclopentadiene. Higher homologs of cyclopentadiene have different rates of formation of the fulvene and thus interfere with the determination. Powell, Edson, and Fisher (*4*) made use of this fact and reported a fulvene method which depends on the differences in rates of formation of the fulvene of cyclopentadiene and methylcyclopentadiene with acetone and benzaldehyde, respectively. No attempt was made to apply their method to the analysis of high purity concentrates.

The exhaustive tube cracking method consists of cracking the dimer concentrates in the vapor phase in a hot tube at 700° F. The hot vapors pass to a still pot, where they are fractionated into arbitrary fractions containing what is assumed to be 100% cyclopentadiene and methylcyclopentadiene, respectively. The material is exhaustively cracked by continuously recycling the still bottoms through the cracking tubes. This method is basically an assay type procedure and was used before the ultraviolet and mass spectrometer methods were applied.

The ultraviolet method which was used in this laboratory is that of Powell and Edson (*3*) and is based on the determination of only cyclopentadiene and methylcyclopentadiene. An isooctane solution of the dicyclopentadiene concentrate is cracked at 355° to 365° C. in a hot wire cracker, followed by measurement of the ultraviolet absorption of a highly diluted portion of the cracked products at 240 and 258 mμ. The concentration of the two components is determined from the solution of two simultaneous equations.

The ultraviolet method as described by Powell and Edson is naturally limited to samples in which no cyclodienes other than cyclopentadiene and methylcyclopentadiene are present. An attempt was made to include the C_7 homolog in a three-component ultraviolet analysis, but no routine use was ever made of this procedure. Aromatics and other materials that are not changed when passed over the cracker do not interfere, as their absorption is corrected for by use of a blank. Any codimers of cyclopentadiene with ultraviolet absorbing materials such as isoprene which crack at 355° C. may cause high results. On the other hand, the presence of an appreciable quantity of these codimers may yield low results due to their failure to crack completely and make the cyclopentadiene available for measurement. The dimers and codimers themselves do not appreciably absorb in the ultraviolet region.

Powell and Edson (*3*) claim an accuracy within about ±0.8% on each component for the ultraviolet method. Analysis of two known mixtures of the pure dimers prepared in this laboratory showed a maximum deviation of ±1.9% from the actual percentage. The data are shown in Table I.

A precision study made in this laboratory with five different analyses has shown a maximum deviation from the mean of ±2.3% for either component and for the total cyclodiene. The standard deviation (σ) was found to be 1.20 for cyclopentadiene, 1.05 for methylcyclopentadiene, and 1.14 for the total (Table II). For samples containing a higher proportion of methylcyclopentadiene, the deviation from the average will probably be greater for the methylcyclopentadiene figure, since the absorption of the methylcyclopentadiene is measured on the side of the absorption band. The presence of the higher homolog (dimethylcyclopentadiene) will also interfere with the analysis.

Table I. Analysis of Known Mixtures by Ultraviolet Absorption Method

Component	Weight, % Synthesis	Weight, % Analysis
Cyclopentadiene dimer	84.8	86.7
Methylcyclopentadiene dimer	15.2	13.3
Total	100.0	100.0
Cyclopentadiene dimer	84.3	85.3
Methylcyclopentadiene dimer	15.7	14.7
Total	100.0	100.0

Table II. Ultraviolet Analysis of Dicyclopentadiene Concentrate

Operator	Cyclopentadiene, Wt. %	Methylcyclopentadiene, Wt. %	Total Purity, Wt. %
1	76.7	12.0	88.7
	73.6	15.4	89.0
	75.6	14.1	89.7
2	75.5	13.9	89.4
	75.5	13.4	88.9
3	78.0	13.7	91.7
	78.1	13.5	91.6
2	74.8	14.4	89.2
3	77.2	14.6	91.8
4	75.4	15.9	91.3
	76.3	15.8	92.1
	74.9	15.8	90.7
3	75.2	15.4	90.6
	76.7	14.2	90.9
5	75.0	14.8	89.8
Average	75.9	14.5	90.4
Std. dev.	1.20	1.05	1.14

Because of the difficulty encountered in complete cracking of all dimers containing cyclopentadiene and the insufficient reproducibility of the ultraviolet method, the mass spectrometer method was developed.

MASS SPECTROMETER METHOD

In the original form of this method, the dimers were cracked in the presence of a diluent in a hot wire cracker at 300° to 400° C. (same as the ultraviolet method) and the cracked products were charged to the mass spectrometer. The diluent, usually isooctane, acted as an internal standard. Some inherent disadvantages of this method are reduced sensitivity because of large excess of inert diluent and necessity for maintaining the cracked products at dry ice temperature to prevent redimerization.

The next modification consisted of heating the entire inlet system of the mass spectrometer to 400° C. The dimer samples were charged to the instrument with an ordinary capillary dipper and were cracked directly in the inlet system. There were several uncontrollable "cold spots" in this system, which probably resulted in incomplete or inconsistent cracking and condensation of heavy components. There were also uncontrollable "hot spots," which resulted in frequent overheating and collapsing of the glass inlet system. In general, reproducibility by this method was not very good.

Dimer samples are now cracked in an external low pressure borosilicate glass cracking chamber which is connected directly to the inlet system of the mass spectrometer. A diagram of the cracker is shown in Figure 1.

In operation the cracker is evacuated to normal inlet system pressure (10^{-3} mm.). A magnetically operated gallium valve located at the exit of the cracker is closed, and the sample is introduced through a heated mercury orifice. After 2 to 5 minutes at 350° to 450° C., the gallium valve is opened and the cracked products are expanded into the main inlet system of the mass spectrometer. The inlet system is constructed so the cracker can be evacuated and charged with a second sample while the first sample is being analyzed.

Both calibration compounds and unknown samples are charged to the cracker on a constant volume basis; therefore, the initial mass spectra which are obtained are expressed in terms of peak height (divisions) per unit volume of material charged. Calibration data have been converted to a basis of peak height per unit weight by dividing the mass spectra of the pure compounds by their respective densities and are thereby made independent of the type (monomer or dimer) of calibration compound used.

The cracking procedure now used gives complete control of cracking temperature and residence time and has materially improved reproducibility. Complete depolymerization of all of the dimers and codimers present is not accomplished even at the low pressure and high temperature used in the cracking step. This was evident from the presence of prominent parent dimer peaks in the mass spectrum of the cracker effluent.

The peaks (m/e) used to determine the individual components are as follows: cyclopentadiene, 65 and 66; isoprene, 68; methylcyclopentadiene, 79 and 80; dimethylcyclopentadiene, 94; cyclopentadiene-acyclic codimer, 134; dipentene, 136; and methylcyclopentadiene-acyclic codimer, 148. The mass spectrum of the unknown sample is converted to a basis of peak height per unit weight by dividing the absolute peak heights by the density of the sample. A sample calculation is presented in Table III.

The heaviest component present is calculated first. For example, the peak height–unit weight of sample at 149 mass divided by the peak height–unit weight of pure codimer at 148 mass represents weight per cent of 148 mass codimer present in the cracked products from the unknown sample. The other components, in order of decreasing molecular weight, are calculated in a like manner after the individual peaks have been corrected for contributions from heavier components.

The calculated values for cyclopentadiene at 65 and 66 peaks normally check within 1%, and an average of the two figures is used. The agreement between the check values for cyclopentadiene and methylcyclopentadiene is taken as evidence of proper calibrations for the heavier components.

The concentration of uncracked cyclic-acyclic codimers indicated by the height of the 134 and 148 peaks is very sensitive to changes in cracking severity. The data in Table IV show that as the cracking severity is increased from 2-minute residence time at 350° C. to 15-minute at 450° C., the amount of uncracked 134 mass codimer decreases from 5.4 weight % to 0.1 weight % and that this decrease is accompanied by proportionate increases in the concentrations of cyclopentadiene and acyclic pentadienes (calculated as isoprene).

It is concluded from this that the codimers which are being used for calibration purposes are representative of the thermally stable codimers which actually exist in the dimer concentrates and that any cyclopentadiene combined in such codimers is theoretically recoverable. It should be remembered, however, that the cracking conditions used in this test approach the ideal and that the actual amount of cyclopentadiene monomer which can be recovered in a commercial process might be considerably lower because of degradation.

The analyses in Table IV have been normalized, as it is believed that essentially all components (99% or better) have been accounted for. The unnormalized totals range from 95 to 105% and on the average are close to 100%. Five minutes' residence time at 450° C. has been found to represent the practical limit of cracking severity for this method. However, from an analytical standpoint, the figures obtained at low cracking severity are just as good as the figures at high severity. Reproducibility and component balance in both cases are very good.

Although this work represents only one application of combined low pressure cracking and mass spectral analysis, modification of the equipment and technique to reach higher temperatures should permit the study of other depolymerization or pyrolysis reactions.

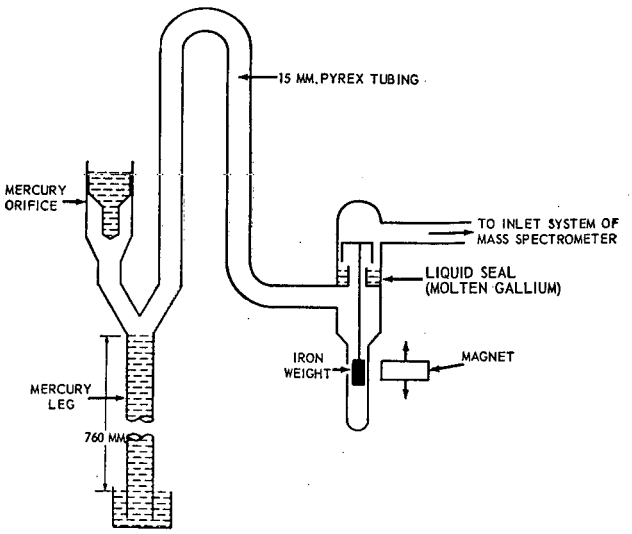

Figure 1. Low pressure cracker for mass spectrometer analysis of cyclopentadiene dimer concentrates

Entire cracking chamber, from mercury orifice to gallium valve, is wrapped with heater wire and insulated

Table III. Sample Calculations

Component	Mass Peak	Peak Height Divisions: Original	Peak Height Divisions: Converted[a]	Calculated Weight, %: Natural	Calculated Weight, %: Normalized
Cyclopentadiene	65	861.00	892.3	75.5	
	66	1920.00	1990.0	74.9	
			Av.	75.2	74.9
Isoprene	68	68.8	71.3	5.9	5.9
Methylcyclopentadiene	79	427.0	442.5	17.4	
	80	246.0	255.0	17.3	
			Av.	17.3	17.3
Dimethylcyclopentadiene	94	5.0	5.2	0.62	0.6
134 mass codimer	134	1.6	1.7	0.27	0.3
Dipentene	136	1.1	1.1	0.94	0.9
148 mass codimer	148	0.5	0.5	0.14	0.1
				100.4	100.0

[a] Original spectrum divided by density of sample, 0.965 gram per cc.

Table IV. Effect of Cracking Severity on Component Balance

Component, Wt. %	Cracking Conditions: 350° C. 2 min.	400° C. 2 min.	450° C. 2 min.	450° C. 5 min.	450° C. 15 min.	400° C. 15 min.	Std. Dev.[a]
Cyclopentadiene	73.7	74.2	74.5	76.1	76.3	75.7	0.20
Methylcyclopentadiene	15.0	15.1	15.5	15.7	15.9	15.9	0.10
Dimethylcyclopentadiene	0.9	0.9	0.9	0.8	0.8	0.8	0.01
Isoprene	2.9	3.5	4.8	5.8	6.1	5.7	0.20
134 mass codimer	5.4	4.4	2.7	0.5	0.1	0.7	0.20
Dipentene (136 mass)	0.8	0.8	0.9	0.9	0.8	1.0	0.30
148 mass codimer	1.3	1.1	0.7	0.2	0.0	0.2	0.05
	100.0	100.0	100.0	100.0	100.0	100.0	

[a] 450° C., 5 minutes.

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RECEIVED for review January 3, 1956. Accepted April 21, 1956. Division of Analytical Chemistry, 128th Meeting, ACS, Minneapolis, Minn., September 1955.

Instrumental Determination of Rates of Peroxide Decomposition in Homogeneous Reactor Fuels

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A conductance method has been developed for following the decomposition of peroxide in aqueous uranyl sulfate solutions at very fast rates which preclude successful use of standard chemical methods. The conductance method, which agrees with the chemical method to within 5%, has been used to investigate catalytic effects from possible corrosion and fission product species present in homogeneous reactor solutions.

A KINETIC study of the decomposition of peroxide in aqueous uranyl sulfate solution has shown the rates to be first order with respect to peroxide (*2*) During the course of this investigation it was found impossible to follow with any precision rates of reaction with half lives of less than 30 seconds, using standard chemical titration methods. Moreover, it was mandatory to develop a method adaptable to remote control operation because of the high radiation intensity of solutions obtained from operating homogeneous nuclear reactors. A conductance method capable of following reactions having half lives of approximately 2 seconds was developed for this purpose.

When hydrogen peroxide is introduced into a uranyl sulfate solution, there is an immediate reaction according to the following equation:

$$UO_2^{++} + H_2O_2 \rightleftarrows UO_4 + 2H^+ \qquad (1)$$

That the hydrogen peroxide reacts essentially quantitatively can be demonstrated by the fact that, in dilute solution, the change in hydrogen ion concentration is consistent with the amount of hydrogen peroxide added and with the stoichiometry of the reaction, whether or not the solubility of the slightly soluble uranyl peroxide is exceeded. In this investigation, however, all experiments were planned so that precipitation was avoided.

The decomposition of peroxide can be represented by two possible alternative mechanisms:

$$\text{A.}\quad UO_4 + 2H^+ = UO_2^{++} + H_2O_2 \qquad (2)$$

$$H_2O_2 \rightarrow H_2O + {}^1/_2\, O_2 \qquad (3)$$

$$\text{B.}\quad UO_4 \rightarrow UO_3 + {}^1/_2\, O_2 \qquad (4)$$

$$UO_3 + 2H^+ \rightarrow UO_2^{++} + H_2O \qquad (5)$$

If the rate-determining step is either Equation 3 or 5 and whether or not the mechanism is A or B, a conductance method for peroxide decomposition should approximate the chemical or titration method, provided a linear relation exists between conductance and hydrogen ion concentration. The fact that the equilibrium in Equation 4 is far to the right increases the sensitivity of the conductance method. Chemical titration, which determines both hydrogen peroxide and uranyl peroxide additively, also does not distinguish between the alternative mechanisms A and B. Dilute solutions (about 8 grams of uranium per liter) were used for the conductance work in order to avoid buffering and nonlinear effects.

EXPERIMENTAL WORK

Apparatus. CHEMICAL METHOD. The reaction vessel for all experiments was a modified three-necked borosilicate glass flask. A motor-driven glass stirrer was inserted through the center opening. Hydrogen peroxide was pipetted through a side opening of the flask into the reacting solution after the latter was brought up to temperature in a thermostat. The other side opening was kept closed during the chemical experiments.

CONDUCTANCE METHOD. A general purpose conductivity bridge with Wagner ground (*3*) built at this laboratory was used for the bulk of the experiments. A Model 200D Hewlett & Packard audio-oscillator supplied a 1000-cycle source. A 1000-ohm Helipot was used for rapid balancing, and a 5-inch DuMont oscilloscope served as the null-point detector. Conductance leads were inserted through the second side opening of the reaction flask, positioned near the edge of the flask and out of the immediate range of the stirrer. "Gray" platinum-bead electrodes (lightly platinized and then heated for several seconds at low red heat), about 0.1 inch in diameter, were placed about 0.5 inch apart in the continuously stirred solution. Several chemical runs were made in the presence and absence of electrodes to check the possibility that the platinum electrodes might accelerate the decomposition of peroxide. No such effect was noted under the conditions of these experiments.

Reagents. Unstabilized 30% hydrogen peroxide, diluted with doubly distilled water, was employed for all experiments. Uranyl sulfate was prepared in the laboratory from the reaction of purified uranium trioxide with sulfuric acid. All other reagents conformed to ACS specifications.

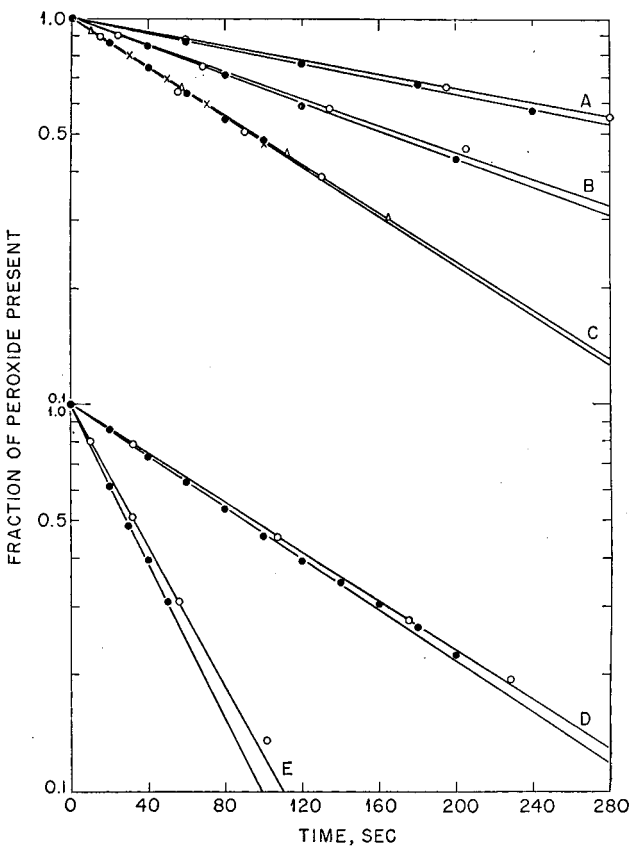

Figure 1. Correlation of chemical and conductance methods

O, △ Titration
●, × Conductance

Procedure. For the chemical method, standard kinetics techniques were employed. Portions of hydrogen peroxide of 0.5 to 1 ml. were rapidly injected into 50 to 100 ml. of uranyl sulfate solution. At various times after addition, samples of the reaction mixture were withdrawn and pipetted into excess ceric sulfate, which was then back-titrated with ferrous ammonium sulfate solution using ferrous *o*-phenanthroline complex as indicator. A sharp end point for the titration was obtained even in the presence of 1*M* uranyl sulfate.

Rate data using the conductance method were obtained by measuring the resistance of the uranyl sulfate solution at various times after the addition of hydrogen peroxide. Immediately after addition, a sharp increase in conductance was noted, which gradually diminished to the value of the original solution as the decomposition of peroxide proceeded to completion. The dilution effects of the hydrogen peroxide added were negligible under the conditions used.

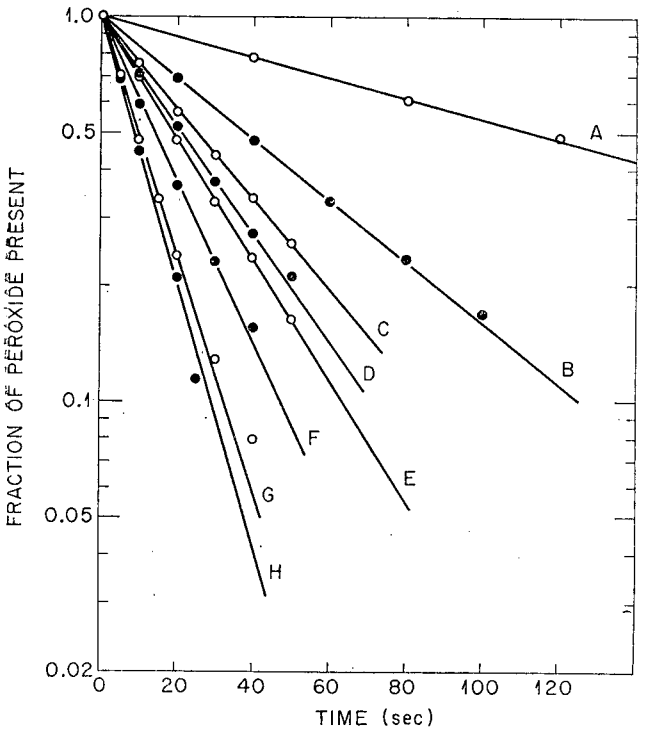

Figure 2. Catalytic effect of iron on peroxide decomposition

A. Uncatalyzed
B. Iron (II), 0.22 p.p.m.
C. 0.45 p.p.m.
D. 0.67 p.p.m.
E. 0.90 p.p.m.
F. 2.0 p.p.m.
G. 4.3 p.p.m.
H. 6.5 p.p.m.

For the catalytic studies, definite concentrations of the various species were added to the reaction flask about 30 seconds before the hydrogen peroxide was added, and conductance readings were taken after allowing enough time for mixing (approximately 30 seconds). Additional catalyst was usually inserted for successive runs until further additions had no effect on the rate of decomposition. Whenever fast rates of decomposition were encountered approximate settings were made on the Helipot, before adding hydrogen peroxide, to take full advantage of the anticipated shift in conductance.

RESULTS AND DISCUSSION

Data for correlating the chemical and conductance methods were obtained in 0.034*M* uranyl sulfate solutions to which hydrogen peroxide was added in an initial concentration of about 0.004 to 0.008*M*.

The conductometric data were usually analyzed by the method of Guggenheim (*1*), except in the case of very fast reactions where only a few experimental points could be obtained.

Some of the data are presented in Table I and Figure 1, where the fraction of peroxide present is plotted against the time in seconds. The designations *A* through *E* on the lines in Figure 1 indicate solutions of varying catalytic activity chosen to determine the correlation over a wide range of rates.

Table I. Comparison of Rate Measurements Determined Simultaneously by Chemical and Instrumental Methods

Solution	Time, sec.	Readings: Chemical	Readings: Conductance	Specific Reaction Rate Constant, ($k \times 10^2$), Sec.$^{-1}$: Chemical	Specific Reaction Rate Constant, ($k \times 10^2$), Sec.$^{-1}$: Conductance
A	60	0.888	0.872	0.21	0.22
	120	0.785	0.770		
	180	0.698	0.673		
$t_{1/2}$ = 312 sec.	240	0.620	0.590		
	300	0.550	0.517		
	360	0.486	0.452		
B	40	0.850	0.843	0.41	0.42
	80	0.725	0.712		
$t_{1/2}$ = 174 sec.	120	0.618	0.603		
	160	0.527	0.510		
	200	0.450	0.430		
C	20	0.867	0.862	0.73	0.74
	40	0.753	0.746		
$t_{1/2}$ = 98 sec.	60	0.653	0.645		
	80	0.568	0.559		
	100	0.492	0.482		
	120	0.428	0.418		
D	20	0.860	0.858	0.74	0.76
	40	0.743	0.734		
$t_{1/2}$ = 94 sec.	60	0.640	0.630		
	80	0.552	0.542		
	100	0.477	0.465		
E	10	0.810	0.790	2.1	2.3
	20	0.655	0.625		
$t_{1/2}$ = 34 sec.	30	0.532	0.490		
	40	0.432	0.389		

The data for experiments *A* through *E* are given in more detail in Table I. A tenfold variation in the specific reaction rate constant was reproduced conductometrically, with an average variation of 5% from the corresponding result obtained by chemical titration. The small difference in rates can probably be attributed to the fact that chemical analysis determines peroxide present both as uranyl peroxide and hydrogen peroxide, whereas the conductometric method indirectly measures only uranyl peroxide formed according to reaction (*1*).

The conductance method was used to study the effect of possible corrosion and fission product species, present in homogeneous reactor solutions, on the rates of decomposition of peroxide. An application of the method is illustrated in Figure 2, indicating how the rate of reaction varies with increasing concentration of catalyst. Approximately a sixtyfold variation in rate is demonstrated. Additional experimental results of the catalytic studies are available elsewhere (*2*).

ACKNOWLEDGMENT

The authors wish to acknowledge their gratitude to R. A. Dandl of the Instrument Division, Oak Ridge National Laboratory, for the design and construction of the modified conductivity bridge used in this investigation, and to C. H. Secoy for his kind encouragement.

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RECEIVED for review January 30, 1956. Accepted March 22, 1956.

Determination of Resin Content of Glass Fiber–Polyester Laminates Containing Calcium Carbonate Filler

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A reasonably accurate determination of the resin content of glass fiber–polyester laminates containing calcium carbonate filler may be made by following the normal procedure for resin content. The values obtained upon ignition are then corrected by treating the ignited residue with ammonium carbonate to convert any reduced calcium oxide back to the carbonate. A quantitative measurement of calcium carbonate cannot be made by treatment of the ignited residue with hydrochloric acid because certain constituents of the glass are dissolved by the acid.

INERT fillers are often used in the production of polyester laminates reinforced with unoriented glass fiber. The most widely used inert filler for this application is precipitated calcium carbonate. Previous work has indicated that the presence of this specific filler introduces appreciable errors in the results obtained by the currently accepted method of determining resin content (*3*). Because the strength properties are, to a certain extent, a function of the resin content, which may vary because of fabrication techniques, the quantitative measurement of the components of plastic laminates reinforced with glass fiber is often necessary to determine whether the materials conform to a specification. Consequently, a modification of the method of test was developed to correct for errors introduced by the calcium carbonate filler. This report describes a reasonably accurate, rapid method for determining the resin content of laminates containing polyester resin, unoriented glass mat, and calcium carbonate filler.

GENERAL CONSIDERATIONS

The resin content of a glass fiber–polyester laminate that does not contain a filler is normally obtained by igniting a specimen to a weight equilibrium in a muffle furnace at 538° to 593° C. and measuring the loss in weight of the specimen. This loss of weight on ignition is then corrected for the loss in weight caused by volatilization or decomposition of the fiber finish, previously determined on samples of the glass fiber, to give the resin content (*3*).

When specimens containing calcium carbonate filler are used, the loss of weight on ignition in the muffle furnace is considerably greater than the sum of resin content plus fiber finish. The excess loss represents carbon dioxide evolved from the filler at temperatures above 525° C. (*1*).

The amount of dissociation of calcium carbonate is dependent upon the temperature and the partial pressure of the carbon dioxide in the furnace atmosphere, and may also be affected by the pyrolysis of the organic matter present in the resin and fiber finish. The dissociation of calcium carbonate into calcium oxide and carbon dioxide is a three-phase, two-component equilibrium system. Application of the phase rule, $P + V = C + 2$, indicates 1 degree of freedom—that is, either temperature or pressure, if arbitrarily fixed, will define the state of equilibrium. Because the normal partial pressure of carbon dioxide in the atmosphere is 0.24 mm., the carbon dioxide contained in the system will be evolved when its partial pressure exceeds that of the carbon dioxide in the atmosphere. This pressure is initially reached at a temperature of approximately 525° C. Consequently, when the temperature is raised above this point, as is required in the method for resin content determination, the carbon dioxide is evolved into the space immediately above the solid constituents, calcium carbonate and calcium oxide. If fresh air is allowed to pass over the specimen, as in a ventilated muffle furnace, some of the carbon dioxide will be drawn out of the crucible and the system of calcium carbonate, calcium oxide, and carbon dioxide will no longer be in equilibrium. More carbon dioxide must be evolved from the calcium carbonate to regain the partial pressure necessary for an equilibrium state. A continuous flow of air, then, results in a continuous evolution of carbon dioxide. The amount of carbon dioxide lost during the period of ignition is dependent not only upon the temperature used but also upon the length of time required to burn the resin.

To regain the weight lost through dissociation of the calcium carbonate, the ignited residue may be treated in some manner to convert the calcium oxide back to calcium carbonate without the formation of other constituents. This conversion may be obtained quantitatively by treating the oxide with an excess of concentrated ammonium carbonate solution. The excess unreacted ammonium carbonate, which dissociates into ammonia, carbon dioxide, and water at 58° C. (*2*), is then removed by evaporating the solution to dryness.

An increase in residual weight obtained with the ammonium carbonate treatment represents quantitatively the amount of carbon dioxide lost on ignition. Subtracting this increase in weight of the ignited residue and the loss in weight arising from the destruction of the fiber finish from the original loss in weight should give a correct value for the original resin content of the plastic laminate.

PREPARATION OF SPECIMENS

Specimens of known composition were prepared using the following materials:

Resin. Selectron 5003, an unsaturated polyester resin; Pittsburgh Plate Glass Co.

Glass Fiber. Unoriented, mechanically bonded glass mat, 2 ounces per square foot, with a chrome size; Bigelow Glass Fiber Division of the Bigelow-Sanford Carpet Co.

Filler. Precipitated calcium carbonate, assay 99.96%; Merck & Co., Inc.

Catalyst. Luperco ATC, 50% benzoyl peroxide and 50% tricresyl phosphate; Lucidol Division, Wallace and Tiernan, Inc., Belleville, N. J.

The composition of the specimens is given in Table I, and duplicates the general range found in some commercial items. The specimens were prepared by adding a predetermined amount of each component to a weighed porcelain crucible to give a total weight of about 3 grams. This weight is approximately that of a glass fiber–polyester laminate specimen 1 by 1 by $\frac{1}{8}$ inch. Quantities of components used were as follows: resin, 50.0%; glass mat (as received), 37.5%; and filler, 12.5% (25.0% based on weight of resin used).

The glass fiber used in this work was obtained from a single piece of mat measuring 2 feet square. The 18 specimens used for the determination of fiber finish content measured approximately 1 by 5 inches; the specimens used in the resin content determination measured approximately 1 by 4 inches for group I and 1 by 3 inches for groups II and III. The average value of 1.5% fiber finish content based on the total weight of glass mat was taken to apply to all of the specimens in the three test groups.

PROCEDURE

The glass mat and calcium carbonate filler were dried at 110° C. for 1 hour to eliminate any traces of moisture, and cooled to

23° C. in a desiccator charged with calcium chloride. The resin was catalyzed with Luperco ATC, 2% by weight of the resin. The components were separately weighed into a porcelain crucible on an analytical balance. The resin was cured at 110° C. for 25 minutes and cooled to room temperature. The specimen was then placed in a muffle furnace at 538° to 593° C. and ignited to constant weight, which required about 1.5 hours. Higher temperatures were avoided to prevent fusion of the glass and the resultant entrapment of unburned carbon particles. Upon removal from the furnace, the specimen was cooled to room temperature in a desiccator and reweighed on an analytical balance. The weight loss on ignition is equal to the weight of the resin, the volatilized or decomposed part of the fiber finish, and any carbon dioxide evolved from the dissociation of the calcium carbonate filler.

The residual material remaining in the crucible was reacted with an excess of a concentrated solution of ammonium carbonate. The specimen was evaporated to dryness at 80° C. to drive off the excess ammonium salt, then heated at 110° C. to constant weight. The specimen was again weighed after cooling in a desiccator to room temperature. The increase in weight obtained from treatment with ammonium carbonate is the weight of the carbon dioxide absorbed by the ignited filler and is equal to the carbon dioxide evolved from the filler during ignition in the furnace.

The resin content in weight per cent is:

$$R = \frac{W_1 - W_2 - W_3 - (W_4 - W_3)}{W_1} \times 100$$

or

$$R = \frac{W_1 - W_2 - W_4}{W_1} \times 100$$

where

W_1 = original weight of specimen, prior to curing
W_2 = weight of fiber finish
W_3 = weight after ignition
W_4 = weight after ammonium carbonate treatment

Following the weighing after the ammonium carbonate treatment, each specimen was transferred from the crucible to a beaker to which 90 ml. of $3N$ hydrochloric acid was added. The acid was heated to 90° C. for 3 to 5 minutes, or until solvent action ceased, and the glass residue was filtered off and repeatedly washed with boiling distilled water until a silver nitrate test of the filtrate was negative for chlorides.

The filter paper containing the acid-insoluble glass fiber residue was placed in a crucible and the filter paper burned off. The specimens were allowed to cool to room temperature in a desiccator prior to the final weighing. Two methods were used to determine the amount of acid-soluble glass. In method A, the soluble constituents were calculated from the difference in weight between the original corrected glass fiber content and the acid-insoluble residue. Values for method B were calculated from the difference in weight between the total acid-soluble constituents and the original calcium carbonate filler.

Table I. Composition of Specimens for Resin Content Determination

Specimen	Original Composition, Weight %[a] Resin[b]	Calcium carbonate filler	Glass fiber, as received	Volatile fiber finish[c]	Glass fiber[d]
		Group I			
1	49.96	None	50.03	0.75	49.28
2	50.02	None	49.98	0.75	49.23
3	49.95	None	50.05	0.75	49.30
4	50.10	None	49.90	0.75	49.15
5	50.02	None	49.98	0.75	49.23
6	49.96	None	50.04	0.75	49.29
		Group II			
7	50.37	12.45	37.18	0.56	36.62
8	49.97	12.58	37.45	0.56	36.89
9	49.86	12.48	37.66	0.56	37.10
10	50.00	12.49	37.51	0.56	36.95
11	50.03	12.50	37.47	0.56	36.91
12	50.19	12.50	37.31	0.56	36.74
		Group III			
13	50.06	12.52	37.42	0.56	36.86
14	50.04	12.54	37.42	0.56	36.86
15	50.13	12.43	37.44	0.56	36.88
16	50.14	12.50	37.36	0.56	36.80
17	49.88	12.63	37.49	0.56	36.93
18	50.22	12.46	37.32	0.56	36.76

[a] Original specimen weights ranged from 2.9974 to 3.0293 grams.
[b] Previously catalyzed with Luperco ATC, 2% by weight of resin.
[c] Based on 1.5% of original weight of glass fiber.
[d] Glass fiber, as received, less volatile fiber finish.

RESULTS

The test results obtained with the ammonium carbonate treatment are given in Table II. Of three groups of specimens tested, group I contained resin and glass, and groups II and III, resin, glass, and calcium carbonate filler. All of the specimens were treated according to the procedure previously outlined, except that the ammonium carbonate treatment was omitted for group III.

The control specimens in group I had a resin content of about 0.01% more than the original resin content after correcting the loss on ignition for fiber finish content. Subsequent treatment with ammonium carbonate gave a measured resin content of about 0.05% less than the original resin content based on the original specimen weight. This indicates that a slight error may be introduced by the retention of a small amount of unvolatilized ammonium carbonate or its products, either adhering to or reacting with the glass fiber or some of its constituents. The difference between measured and original resin content results from an average weight increase of about 2 mg. after the ammonium carbonate treatment.

Table II. Resin Content Determination[a]

(All results are weight per cent)

Specimen	Loss of Weight on Ignition	Fiber Finish	Corrected Ignition Weight Loss[b]	Carbon Dioxide Absorbed[c]	Resin Content Measured[d]	Resin Content Original	Diff. in Resin Content, Measured less Original
				Group I			
1	50.68	0.75	49.93	0.07	49.86	49.96	−0.10
2	50.84	0.75	50.09	0.06	50.03	50.02	0.01
3	50.61	0.75	49.86	0.07	49.79	49.95	−0.16
4	50.87	0.75	50.12	0.04	50.08	50.10	−0.02
5	50.97	0.75	50.22	0.12	50.10	50.02	0.08
6	50.61	0.75	49.86	0.04	49.82	49.96	−0.14
Av.	50.76	0.75	50.01	0.06	49.95	50.00	−0.05
				Group II			
7	52.72	0.56	52.16	1.79	50.37	50.37	0.00
8	53.17	0.56	52.61	2.73	49.88	49.97	−0.09
9	55.64	0.56	55.08	5.11	49.97	49.86	0.11
10	55.38	0.56	54.82	4.78	50.04	50.00	0.04
11	51.82	0.56	51.26	1.28	49.98	50.03	−0.05
12	55.52	0.56	54.96	4.73	50.23	50.19	0.04
Av.	54.04	0.56	53.48	3.40	50.08	50.07	0.01
				Group III			
13	53.96	0.56	53.40	..	53.40	50.07	3.33
14	56.02	0.56	55.46	..	55.46	50.04	5.42
15	53.55	0.56	52.99	..	52.99	50.13	2.82
16	55.32	0.56	54.76	..	54.76	50.14	4.61
17	52.05	0.56	51.49	..	51.49	49.88	1.61
18	55.42	0.56	54.86	..	54.86	50.22	4.64
Av.	54.39	0.56	53.83	..	53.83	50.08	3.75

[a] All values based on original weight of specimen.
[b] Loss of weight on ignition less fiber finish; results obtained by normal method of determining resin content.
[c] Increase in residual weight after treatment with ammonium carbonate.
[d] Corrected ignition weight loss less amount of carbon dioxide absorbed.

After the ammonium carbonate treatment the difference between the measured resin content and original resin content for the specimens in group II was about 0.01% based on the original weight of resin. The difference in resin content in both groups I and II appears to be a measure of the variation in glass fiber finish content rather than errors in the resin content determination. These values fall within the range that could be expected from the data obtained on the fiber finish content of the glass fiber.

The specimens in group III were not treated with ammonium carbonate. The measured loss on ignition, after correcting for the fiber finish content, was about 3.75% more than the original resin content based on the original specimen weight. This error is attributed to the loss in weight of the calcium carbonate filler caused by evolution of carbon dioxide.

The ignited residues of the three groups of specimens were treated with hot 3*N* hydrochloric acid, a relatively mild acid treatment that was neither carried to completion nor intended to indicate the limiting solubility of glass in hydrochloric acid. This treatment was primarily designed to remove the filler quantitatively, thus allowing a quantitative determination of the glass fiber present. Such a method, if applicable, would provide a means of obtaining a total analysis of the material. Values obtained from this treatment, however, indicate that a considerable amount of the glass fiber was dissolved by the acid along with the calcium carbonate and calcium oxide. This indicates the effect of even mild acid treatment on the glass, and of the magnitude of error in this method which is now being used by some workers in the field.

The amount of acid-soluble glass is reported in Table III and was determined by two methods, *A* and *B*. In method *A*, the amount of acid-soluble glass was determined by the difference in weight of the acid-insoluble residue from the original amount of glass fiber present. Average values obtained for groups I and II were 8.6 and 8.3% acid-soluble glass, respectively. Group III, which was not subjected to the ammonium carbonate treatment, had an average loss of 14.2%.

The loss in weight determined by method *B* was made on the assumption that all of the original calcium carbonate was present as such in the residue and was dissolved by the acid. The difference between the total weight of acid-soluble material and the amount of calcium carbonate originally present was considered to be the amount of acid-soluble glass. Results obtained for groups I and II were 8.7 and 8.3%, respectively, comparing favorably with results obtained by method *A*. The average weight loss for group III was 4.0%. The difference between this value and the similar one obtained by method *A* results from the fact that a certain amount of the calcium carbonate had been reduced to calcium oxide and use of the original weight of the calcium carbonate introduces an error, the magnitude of which is determined by the amount of carbon dioxide evolved during ignition.

The volatile part of the finish on the glass fiber was obtained according to the method used for determination of resin content (*3*). The loss in weight resulting from ignition in a muffle furnace under the usual conditions is assumed to be the volatile part of the fiber finish and is reported in Table IV as weight per cent of the original glass fiber. Results obtained on the 2-ounce mat in this and previous work have indicated a wide variability of the amount of finish not only from roll to roll but also from location to location within a roll. The results given in Table IV indicate that the variation obtained is high.

The variation in fiber finish may introduce an error in the measured resin content, the magnitude of which is dependent upon the uniformity of the fiber finish and the proportions of glass and resin used in the laminate.

Although this method provides for a quantitative determination of the resin content, it does not provide for a quantitative determination of the glass fiber and calcium carbonate filler in a sample of unknown composition. Because a total analysis of a specimen is sometimes useful, the following modification of the described method is proposed as one that might be used successfully for the determination of glass fiber and calcium carbonate.

The specimen is ignited at 538° to 593° C. until the carbonaceous material resulting from pyrolysis of the resinous materials is removed. The furnace temperature is then raised to 1000° C. to convert all of the calcium carbonate to calcium oxide. The weight increase of the residue after ammonium carbonate treatment is a quantitative measurement of the carbon dioxide content of the calcium carbonate. Knowledge of this value permits calculation of the original amount of calcium carbonate. The amount of glass fiber present is then obtained from the difference in weight of the original specimen less the sum of the weights of

Table III. Determination of Acid-Soluble Constituents of Glass in Glass Fiber–Polyester Plastics

(All results are weight per cent)

Specimen	Method *A*[a] Glass fiber content[c,e]	Acid-insoluble glass[e]	Acid-soluble glass[e]	Acid-soluble glass[f]	Method *B*[b] Acid-soluble glass and filler[e]	Filler content[d,e]	Acid-soluble glass[e]	Acid-soluble glass[f]
				Group I				
1	49.28	45.11	4.17	8.47	4.28	None	4.28	8.69
2	49.23	45.61	3.62	7.35	3.61	None	3.61	7.33
3	49.30	46.43	2.87	5.82	3.03	None	3.03	6.14
4	49.15	43.85	5.30	10.78	5.32	None	5.32	10.83
5	49.23	44.05	5.18	10.51	5.10	None	5.10	10.37
6	49.29	44.97	4.32	8.76	4.46	None	4.46	9.04
Av.	49.25	45.00	4.25	8.62	4.30	...	4.30	8.73
				Group II				
7	36.62	34.13	2.49	6.79	14.93	12.45	2.48	6.79
8	36.89	33.84	3.05	8.28	15.72	12.58	3.14	8.52
9	37.10	34.57	2.53	6.82	14.91	12.48	2.43	6.55
10	36.95	32.52	4.43	11.98	16.88	12.49	4.39	11.87
11	36.91	34.50	2.41	6.50	14.96	12.50	2.46	6.64
12	36.74	33.26	3.48	9.47	15.94	12.50	3.44	9.36
Av.	36.87	33.80	3.07	8.31	15.56	12.50	3.06	8.29
				Group III				
13	36.86	30.50	6.36	17.25	15.54	12.52	3.02	8.21
14	36.86	29.80	7.06	19.16	14.18	12.54	1.64	4.44
15	36.88	34.48	2.40	6.51	11.96	12.43	−0.47	−1.27
16	36.80	28.71	8.09	21.97	15.97	12.50	3.47	9.44
17	36.93	33.03	3.90	10.56	14.92	12.63	2.29	6.22
18	36.76	33.30	3.46	9.42	11.28	12.46	−1.18	−3.20
Av.	36.85	31.64	5.21	14.15	13.98	12.51	1.47	3.97

[a] Acid-soluble glass calculated from original corrected glass fiber content less residue insoluble in hydrochloric acid.
[b] Acid-soluble glass calculated from loss of weight on treatment with hydrochloric acid less original filler content.
[c] Original glass content less volatile fiber finish.
[d] Original calcium carbonate content.
[e] Based on original weight of specimen.
[f] Based on weight of glass.

Table IV. Volatile Glass-Fiber Finish Content

Area Sampled	No. of Specimens	Volatile Fiber Finish Content, Wt. % Av.[a]	Std. dev.	Range
1 sq. ft.	8[b]	2.06	0.11	1.92 to 2.23
4 sq. ft.	18[b]	1.51	0.11	1.28 to 1.66
125-yd. roll	20[b]	1.34	0.25	0.79 to 1.94
125-yd. roll	20	1.52	0.36	0.93 to 2.26

[a] Average loss in weight after ignition in muffle furnace.
[b] These specimens obtained from the same 125-yard roll of glass mat.

calcium carbonate and resin. The volatile fiber finish is included in the values for resin content and could only be estimated.

The results presented (*a*) indicate the magnitude of the error introduced by calcium carbonate decomposition on results obtained using normal analytical ignition procedures for the determination of resin content, and (*b*) provide a simple, but precise, technique for correcting the resultant errors. The method described is useful in those cases where resin content is limited by specification, and where failure to correct for the loss of carbon dioxide would result in the rejection of the material for its specified use, and where fairly accurate resin contents are needed.

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RECEIVED for review May 4, 1955. Accepted April 19, 1956. The work reported here was sponsored by the Materials Laboratory, Directorate of Research, Wright Air Development Center, U.S.A.F., under Contract No. 33(038)51-4060.

End Point Calculation in Conductometric and Photometric Titrations

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An objective method of end point calculation is described for conductometric or photometric titration curves which have no sharp end-point indicating change of slope. These include all titrations which would be difficult or impractical by the potentiometric method. The method makes use of the curved part of the titration curve near the equivalence point and minimizes end point errors due to variations in the equivalent conductivities, deviations from Beer's law, and systematic experimental errors. An end point accuracy of better than 0.5% is obtained even in cases where the end point error may be as large as 10% by other methods of calculation. No additional data are required.

CONDUCTOMETRIC and photometric methods of titration can give accurate results in many cases where the potentiometric method fails because the inflection point on the plot of electromotive force *vs.* volume of titrant is not clearly defined. For example, the photometric titration of *p*-bromophenol (pK_A 9.2) with aqueous sodium hydroxide can be accurate to better than 1% at concentrations as low as 0.001*M* (*3*, *4*). Similarly, 0.001*M* *p*-nitrophenol (pK_A 7.0) can be titrated photometrically in the presence of 0.001*M* *m*-nitrophenol (pK_A 8.3) with an accuracy better than 2% (*3*).

However, in all cases where the potentiometric method fails, the conductometric and photometric titration curves are also difficult to interpret because there is no sharp change of slope at the equivalence point. The difficulties are illustrated by the titration of 0.01*N* aqueous sodium acetate with 1*N* hydrochloric acid, a titration which would be difficult by the potentiometric method. The conductometric titration curve in water at 25° C. is shown in Figure 1. The ordinate, κ, is the conductivity, and the abscissa, ϵ, the ratio of the equivalents of titrant to those of substrate. The curve was computed using the known values of the dissociation constants and equivalent conductivities at the ionic strengths existing during the titration (*7*). As the figure shows, there is no sharp end-point indicating change of slope at the equivalence point. However, there are two nearly linear segments, one before and the other past the equivalence point. The end point is usually taken as the intersection of the two straight lines drawn through the points in these linear ranges.

Under certain idealized conditions (no changes in the equivalent conductivities and no volume changes during the titration), this procedure has a sound basis in theory. There is a linear range before the equivalence point where the added titrant reacts quantitatively with the substrate; there is a linear range past the equivalence point where the added titrant no longer reacts with the substrate, the substrate-titrant reaction being complete; and the intersection of the two straight lines defined in this way occurs at the equivalence point.

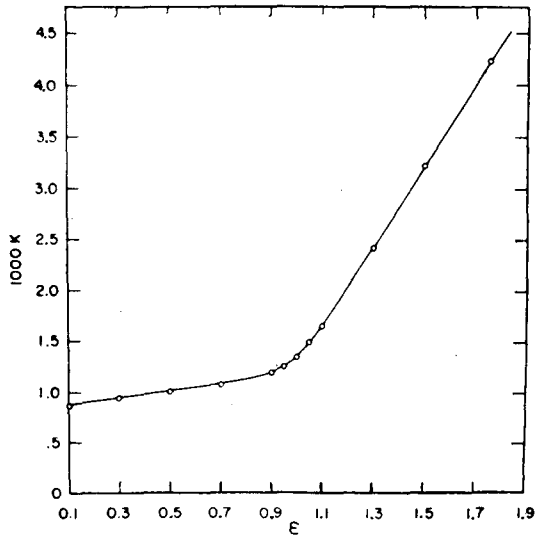

Figure 1. Conductometric titration of 0.01*N* sodium acetate with 1*N* hydrochloric acid in water at 25° C.

Unfortunately, it is difficult in practice to recognize these linear ranges. For example, in Figure 1, only the portion of the titration curve between $\epsilon = 0.9$ and $\epsilon = 1.1$ has pronounced curvature. Because of experimental error, different portions of the nearly linear remainder will appear to be linear in different titrations, and the end point is sensitive to the ϵ-range selection which is made. As shown in Table I, errors of the order of 2% may arise from this cause. (This error is, of course, in addition to that caused by the uncertainty of the experimental data.) Moreover, the actual choice of the linear ranges is subjective.

To make the selection of the linear ranges objective, one possi-

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ble approach is to precalculate their positions from theory. This approach was successful in the recent work on photometric titration (*3*), but in general is subject to serious limitations. First, the relevant equilibrium constants and substrate concentrations must be known in advance, at least approximately. Second, because the linear ranges lie well before and past the equivalence point, progressive changes in the equivalent conductivity or progressive deviations from Beer's law during the titration can cause very large errors. This second limitation is illustrated by the conductometric titration of 0.01*N* ammonia with 1*N* acetic acid in anhydrous methanol at 18° C., Reaction 1.

$$NH_3 + CH_3COOH = NH_4^+ + CH_3COO^- \qquad (1)$$

The relevant data are shown in Table II, and the titration curve is plotted in Figure 2. [The symbol K is used in this paper to denote thermodynamic equilibrium constants, and k to denote equilibrium constants based on molar concentrations (*6*).] The data in column 4 of Table II show that more than 98% of the added titrant is protolyzed at $\epsilon \leqslant 0.5$, and that the proton transfer to the ammonia is better than 97% complete when $\epsilon \gg 1.5$. (There is some dilution during the titration.) Accordingly, the linear ranges might be selected as $0 < \epsilon < 0.5$, and $\epsilon \gg 1.5$.

Table I. Dependence of End Point on ϵ-Range Selection

(Conductometric titration of 0.01*N* sodium acetate with 1*N* hydrochloric acid in water at 25 °C.)

ϵ-Range Selection	ϵ at End Point[a]
0.1–0.5; 1.5–2.0	0.984
0.1–0.5; 1.1–1.5	0.974
0.5–0.9; 1.5–2.0	1.007
0.5–0.9; 1.1–1.5	0.997

[a] For calculations, straight lines were fitted to points of Figure 1 in each of the linear ranges by method of least squares; intersections were computed analytically.

However, when straight lines are fitted to the data in these ranges, the intersection occurs at $\epsilon = 0.898$, the end point error being over 10%. By far the greater part of this error is due to the variation of the equivalent conductivity, Λ. When the same data are recalculated on the incorrect assumption that Λ is constant at 72.6 (which is the value at the equivalence point), the end point lies at $\epsilon = 0.974$.

Table II. Conductometric Titration of 0.01*N* Ammonia with 1*N* Acetic Acid in Methanol at 18° C.[a]

ϵ	$10^{11}k_A$ Acetic acid	$10^{11}k_A$ Ammonium ion	$10^2 c_{NH_4^+}$	Λ	$10^3\kappa$
0.1	28.9	0.74	0.100	90.8	0.091
0.3	34.8	0.74	0.297	82.8	0.246
0.5	39.4	0.74	0.488	78.7	0.384
0.7	43.4	0.74	0.669	75.4	0.504
0.9	46.7	0.74	0.822	73.3	0.603
1.0	47.8	0.74	0.881	72.6	0.640
1.1	48.6	0.74	0.916	72.3	0.662
1.3	49.1	0.74	0.945	71.8	0.679
1.5	49.5	0.74	0.957	71.6	0.685
1.75	49.6	0.74	0.962	71.6	0.689
2.0	49.7	0.74	0.965	71.5	0.690

[a] Based on the following data: $K_A = 2.24 \times 10^{-10}$ for acetic acid, 7.4×10^{-12} for ammonium ion (*1*, *2*); Λ of ammonium ion and acetate ion as a function of the ionic strength, μ, $\log \Lambda = 2.049 - 0.911\,\mu^{1/3}$ (*2*); k_A values were calculated from K_A and interionic attraction theory.

In order to minimize errors of the type just illustrated, the new method uses only points near the equivalence point, even though located on the curved part of the titration curve. The method is based on the fact that, for any two points on the titration curve below $\epsilon = 1$, there exist two conjugate points above $\epsilon = 1$ (actually, an infinite set of conjugate points) such that the straight lines drawn through the two points below $\epsilon = 1$ and through the two conjugate points above intersect exactly at $\epsilon = 1$. The ϵ-values of these conjugate points can be calculated when an analytical expression for the titration curve has been obtained. In the general case, the ϵ-values depend in a complicated way on the substrate concentration and on the equilibrium constant for the substrate-titrant reaction. However, in the important special case where substrate and titrant react in 1 to 1 molar amounts —that is, in all acid-base titrations and in many precipitation and oxidation-reduction processes—the ϵ-values turn out to be independent of concentration and equilibrium constant, and their calculation is a simple matter.

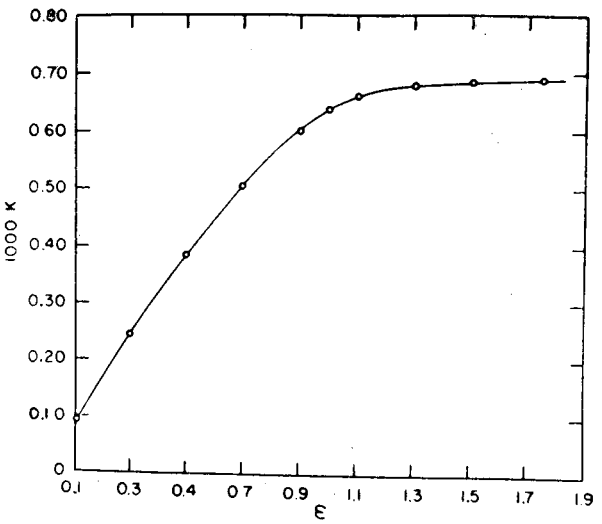

Figure 2. Conductometric titration of 0.01*N* ammonia with 1*N* acetic acid in methanol at 18° C.

Given the ϵ-values of four conjugate points and a crude first estimate of the end point, the correct end point is calculated by successive approximations.

This paper presents the theoretical basis of this method, and examples of its application to various cases.

THEORY OF THE METHOD

General Equation. Consider a physical property G whose variation with solute concentration is given by the linear Equation 2, where the summation $\Sigma g_i c_i$ extends over all solutes.

$$G = G_o + \Sigma g_i c_i \qquad (2)$$

It can be shown, by expanding G in a Taylor series about the equivalence point, that in any given titration Equation 2 is always valid near $\epsilon = 1$. G_o may, but need not, be the value of G for the pure solvent. The coefficients g_i are constants characteristic of each solute and equal to $\delta G/\delta c_i$ at the equivalence point. For a single titration, each c_i and hence G are functions of the single variable ϵ.

It is desired to pick two points (G_1, ϵ_1) and (G_2, ϵ_2) in the range $\epsilon < 1$, and then find two conjugate points (G_3, ϵ_3) and (G_4, ϵ_4) in the range $\epsilon > 1$ such that the straight lines represented by Equations 3 and 4 intersect exactly at $\epsilon = 1$.

$$(G - G_1)/(\epsilon - \epsilon_1) = (G_2 - G_1)/(\epsilon_2 - \epsilon_1) \qquad (3)$$

$$(G - G_3)/(\epsilon - \epsilon_3) = (G_4 - G_3)/(\epsilon_4 - \epsilon_3) \qquad (4)$$

This imposes the restraint,

$$[G_2(1 - \epsilon_1) - G_1(1 - \epsilon_2)]/(\epsilon_2 - \epsilon_1) = [G_4(1 - \epsilon_3) - G_3(1 - \epsilon_4)]/(\epsilon_4 - \epsilon_3) \quad (5)$$

Because Equation 5 is the sole restraint upon the four points, three of the points are independent. The fourth can be computed from Equation 5 when G is known as a function of ϵ.

Weak Base Plus Weak Acid. Equation 5 will now be applied to a specific example, the titration of ammonia whose initial concentration is b, with acetic acid whose concentration is so large that volume changes during the titration can be neglected. At a given value of ϵ before the equivalence point, the concentrations of ammonia, acetic acid, and ammonium acetate are, respectively, $[b(1 - \epsilon) + x]$, x, and $(b\epsilon - x)$. By use of Equation 2, G is given by

$$G = G_o + g_1 b + g_2 b\epsilon - \delta g x \quad (6)$$

where

$$g_1 = g_{NH_3}$$
$$g_2 = g_{NH_4Ac} - g_1$$
$$\delta g = g_2 - g_{HAc}$$

Past the equivalence point, the concentrations of ammonia, acetic acid, and ammonium acetate are, y, $[(\epsilon - 1)b + y]$, and $(b - y)$, respectively. Therefore,

$$G = G_o + g_3 b + g_4 b\epsilon - \delta g y \quad (7)$$

where

$$g_3 = g_{NH_4Ac} - g_4, \text{ and } g_4 = g_{HAc}$$

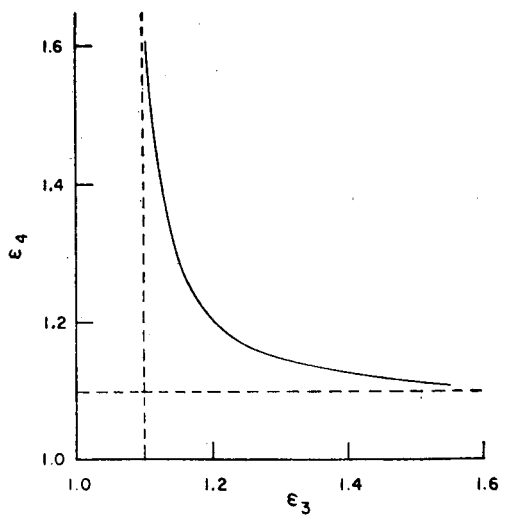

Figure 3. Plot of ϵ_3 *vs.* ϵ_4

$\epsilon_1 = 0.5$; $\epsilon_2 = 0.9$

Now x and y must be expressed as functions of ϵ. This is done by means of the equilibrium constant for Reaction 1—namely,

$$\frac{(b\epsilon - x)^2}{x[b(1 - \epsilon) + x]} = \frac{(b - y)^2}{y[(\epsilon - 1)b + y]} = k_P \quad (8)$$

Equation 8 may be simplified on the following basis. The terms $\delta g x$ in Equation 6 and $\delta g y$ in Equation 7 are the deviations of the (G,ϵ) plots from a straight line. Except very close to $\epsilon = 1$, these deviations are small, and x can be approximated with sufficient accuracy by $b\epsilon^2/(1 - \epsilon)k_P$, and y by $b/(\epsilon - 1)k_P$. The resulting expressions for G are:

$$\epsilon < 1: \quad G = G_o + g_1 b + g_2 b\epsilon - \delta g b\epsilon^2/(1 - \epsilon)k_P \quad (9)$$

$$\epsilon > 1: \quad G = G_o + g_3 b + g_4 b\epsilon - \delta g b/(\epsilon - 1)k_P \quad (10)$$

Equation 9 applies to the left-hand side of Equation 5, and Equation 10 to the right-hand side. When the mathematical operations required by Equation 5 are carried out, all terms and factors involving the b, k_P, or g_i values can be eliminated, and this result is obtained:

$$\frac{\epsilon_2 + \epsilon_1 - 2\epsilon_1\epsilon_2}{(1 - \epsilon_2)(1 - \epsilon_1)} = \frac{\epsilon_4 + \epsilon_3 - 2}{(\epsilon_3 - 1)(\epsilon_4 - 1)} \quad (11)$$

Equation 11 contains no parameters characteristic of the specific example given; therefore, it is a general result and will apply equally to any other titration of a weak base with weak acid or of a weak acid with weak base.

According to Equation 11, for any two points ϵ_1 and ϵ_2 there is an infinite set of conjugate points ϵ_3 and ϵ_4, because either ϵ_3 or ϵ_4 is still independently variable. The variation of ϵ_3 with ϵ_4 for the specific case $\epsilon_1 = 0.5$, $\epsilon_2 = 0.9$ is shown in Figure 3. In order to minimize the end point error, it is advisable to choose ϵ_3 and ϵ_4 not too far from the equivalence point, yet far enough apart to define a straight line with satisfactory accuracy.

Table III. End Points in Conductometric Titration of 0.01*N* Ammonia with 1*N* Acetic Acid in Methanol at 18° C.

ϵ_1	ϵ_2	ϵ_3	ϵ_4	End Point, ϵ
According to Equation 11; points near $\epsilon = 1$				
0.5	0.9	1.5	1.125	1.004
0.7	0.95	1.3	1.055	0.995
0.5	0.8	1.5	1.33	1.000
According to Equation 11; increasing distance from $\epsilon = 1$				
0.5	0.9	1.5	1.125	1.004
0.3	0.7	2.0	1.57	0.967
0.3	0.5	3.3	2.0	0.947
Effect of deviations from Equation 11				
0.5	0.9	1.5	1.11	1.000
0.5	0.8	1.5	1.11	0.977
0.6	0.9	1.5	1.11	1.006
0.5	0.9	1.4	1.11	0.998
0.5	0.9	1.5	1.21	1.019

The application of Equation 11 will now be illustrated by the data, which are listed in Table II, for the titration of 0.01*N* ammonia with 1*N* acetic acid in methanol. Because three of the four points may be chosen independently, let us choose $\epsilon_1 = 0.5$, $\epsilon_2 = 0.9$, and $\epsilon_3 = 1.5$. The value of ϵ_4 is then obtained from Equation 11 as 1.125. The corresponding values of $10^3\kappa$ are 0.384, 0.603, 0.685, and 0.666, and the equations of the two conjugate straight lines are

$$10^3\kappa = 0.110 + 0.548\epsilon$$
$$10^3\kappa = 0.609 + 0.051\epsilon$$

The intersection lies at $\epsilon = 1.004$. Other end points computed by the same method are shown in Table III.

The end points based on Equation 11 are remarkably accurate when the points are fairly close to $\epsilon = 1$. As the distance from $\epsilon = 1$ increases, the end point error becomes larger because of increasing deviations from Equation 2. However, by proper choice of the four points, an accuracy of better than 0.5% is readily attainable. This is the more remarkable when it is remembered that the titration is difficult not only because Λ varies, but also because the equilibrium constant for Reaction 1 is relatively small. At the equivalence point, $k_P = 65$. In order for a titration of this type to be successful by potentiometric methods, k_P should be at least 10^4. The present method, therefore, extends the range of titratability to much lower k_P values without substantial loss in accuracy.

Table III also shows the sensitivity of the end point to errors in the ϵ-values. In general, the sensitivity increases with the curvature at ϵ_1, ϵ_2, ϵ_3, or ϵ_4. However, it appears that errors of the order of 0.01 in ϵ can usually be tolerated.

Because of the approximations made in expressing x and y as functions of ϵ in Equations 6 to 10, none of the four points should be chosen very close to 1.000. In practice, the accuracy is satisfactory if the range $0.95 < \epsilon < 1.05$ is excluded.

Weak Base plus Strong Acid. Consider the titration of sodium acetate whose initial concentration is a, with hydrochloric acid whose concentration is so large that volume changes during the titration can be neglected. If x equals the concentration of hydrogen ion before the equivalence point, and y is the concentration of acetate ion past the equivalence point, these equations are obtained.

$$\epsilon < 1: \quad G = G_o + g_5 a + g_6 a\epsilon - \delta g x \quad (12)$$

$$\epsilon > 1: \quad G = G_o + g_7 a + g_8 a\epsilon - \delta g y \quad (13)$$

where

$$g_5 = g_{NaAc}$$
$$g_6 = g_{HAc} + g_{NaCl} - g_5$$
$$g_7 = g_{NaCl} - g_{HCl} + g_8$$
$$g_8 = g_{HAc}$$
$$\delta g = g_8 - g_{H^+Ac^-}$$

According to the same approximation shown previously $x = k_A\epsilon/(1 - \epsilon)$, and $y = k_A/(\epsilon - 1)$. Using these values in Equations 12 and 13 and substituting in Equation 5, Equation 14 is obtained as the restraint upon ϵ_1, ϵ_2, ϵ_3, and ϵ_4.

$$\frac{1 - \epsilon_1\epsilon_2}{(1 - \epsilon_2)(1 - \epsilon_1)} = \frac{\epsilon_4 + \epsilon_3 - 2}{(\epsilon_3 - 1)(\epsilon_4 - 1)} \quad (14)$$

Again all parameters characteristic of the specific example have disappeared, and Equation 14 is therefore applicable to any titration of a weak base with strong acid, or of a weak acid with strong base.

When Equation 14 is applied to the end point determination for the titration of 0.01N sodium acetate with 1N hydrochloric acid (for which the titration curve is shown in Figure 1), the results shown in Table IV are obtained, which demonstrate that an accuracy of 0.3% is possible. This titration would have been very difficult by the potentiometric method.

Table IV. End Points in Conductometric Titration of 0.01N Sodium Acetate with 1N Hydrochloric Acid in Water at 25° C.

ϵ_1	ϵ_2	ϵ_3	ϵ_4	End Point, ϵ
0.5	0.9	1.4	1.12	1.003
0.5	0.9	1.5	1.11	1.002
0.5	0.8	1.4	1.29	1.003
0.7	0.9	1.3	1.11	1.000

Other Cases. The method just described is readily extended to other types of titration. The fundamental equation is 5, and G must be known as a function of ϵ. The relationship of G to ϵ can be expressed simply and with sufficient accuracy as follows. At $\epsilon < 1$, the concentration, x, of unreacted titrant, and at $\epsilon > 1$, the concentration, y, of unreacted substrate, are computed from the appropriate equilibrium expressions by neglecting the effect of incomplete reaction on the other concentrations. If, moreover, Equation 2 is valid (this is always the case near the equivalence point), Equation 5 reduces to the simpler form of Equation 15.

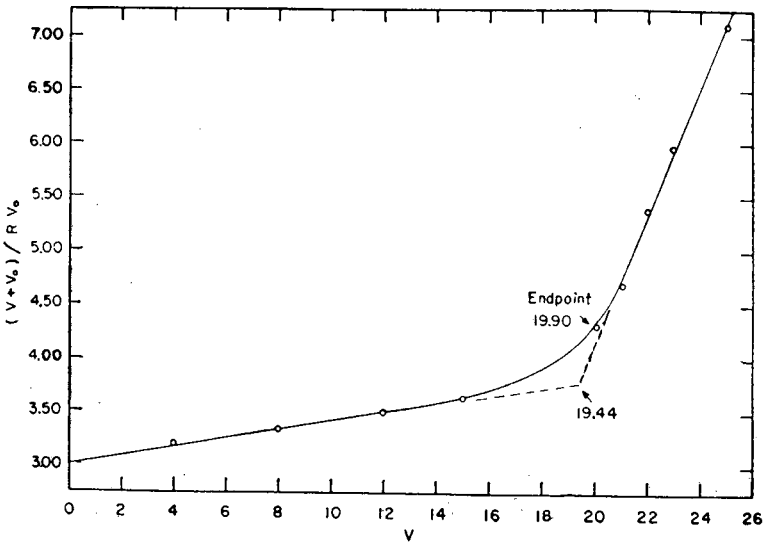

Figure 4. Conductometric titration of 0.008N sodium azide with 0.1N hydrochloric acid in water

$$[x_2(1 - \epsilon_1) - x_1(1 - \epsilon_2)]/(\epsilon_2 - \epsilon_1) = [y_4(1 - \epsilon_3) - y_3(1 - \epsilon_4)]/(\epsilon_4 - \epsilon_3) \quad (15)$$

When the substrate and titrant react in 1 to 1 molar amounts, the equation among ϵ_1, ϵ_2, ϵ_3, and ϵ_4 does not involve any parameters characteristic of the specific titration, as shown by the following general result. Let the titration be represented by the chemical Equation 16.

$$A + B = mC + nD \quad (16)$$

The relationship among the four ϵ-values is then found to be

$$\frac{\epsilon_2{}^{m+n}(1 - \epsilon_1)}{(\epsilon_2 - \epsilon_1)(1 - \epsilon_2)} - \frac{\epsilon_1{}^{m+n}(1 - \epsilon_2)}{(\epsilon_2 - \epsilon_1)(1 - \epsilon_1)} = \frac{\epsilon_4 + \epsilon_3 - 2}{(\epsilon_3 - 1)(\epsilon_4 - 1)} \quad (17)$$

When the stoichiometry is 1 to 1 but two or more substrates are present, the relationship among ϵ_1, ϵ_2, ϵ_3, and ϵ_4 involves also the ratios of their concentrations. For example, if the weak acid HA (initial concentration a) is titrated with strong base in the presence of a weaker acid HB (initial concentration b) the relationship among the four ϵ-values (ϵ now being defined as equivalents of titrant per equivalent of HA) can be shown to be

$$\frac{1 - \epsilon_1\epsilon_2}{(1 - \epsilon_2)(1 - \epsilon_1)} = \frac{\epsilon_4 + \epsilon_3 - 2}{(\epsilon_4 - 1)(\epsilon_3 - 1)} - \frac{a}{b} \quad (18)$$

When the stoichiometry is not 1 to 1, Equation 15 is still applicable, but the final expressions involve the substrate concentration and the equilibrium constant for the substrate-titrant reaction. The method is still useful, but approximate values of these quantities must be known.

APPLICATION OF METHOD

Convergence of End Point Calculations. When the present method is applied, a graph of the titration curve is constructed and a tentative estimate of the end point is obtained by conventional procedures. By use of this estimate and a convenient set of ϵ_1, ϵ_2, ϵ_3, and ϵ_4 values, four points are located on the titration curve, the two conjugate straight lines are constructed, and a second estimate of the end point is obtained. This process is repeated until successive estimates converge.

The data in Table III show clearly that the convergence will be best if the four points are chosen so that they lie outside the region where there is great curvature. Even a rather large error in the ϵ-values will then produce only a small error in the end point. This effect is illustrated by the following data for the

titration of 0.01N ammonia with 1N acetic acid in methanol at 18° C. If the four ϵ-values are taken as 0.5, 0.9, 1.5, and 1.125 and if the first end point lies at $\epsilon = 0.900$, successive end points will be 0.932, 0.953, 0.967, 0.975. . . . On the other hand, if the four ϵ-values are taken somewhat further from the equivalence point as 0.5, 0.8, 1.5, and 1.33, successive end points will be 0.900, 0.962, 0.985, 0.992, 0.998. For the titration of 0.01N sodium acetate with 1N hydrochloric acid in water at 25° C., if the ϵ-values are taken as 0.5, 0.8, 1.4, and 1.29, successive end points will be 0.900, 0.986, 1.004. In this case the convergence is very satisfactory.

These calculations point out the practical limits of the present method. As the change in slope at the equivalence point becomes less sharp and the curved part of the titration curve widens, the accuracy of the first end point estimate and the convergence of successive estimates both become poor. The two effects work in concert, and in cases of very gradual change of slope the calculation becomes prohibitively long. However, even in these cases the convergence limit of successive end points is very close to the equivalence point.

In the titration of sodium acetate the first estimate of the end point is not likely to be in error by more than 3% (Table I). A single calculation suffices to find the final end point. This may then be verified with a second set of ϵ_1, ϵ_2, ϵ_3, and ϵ_4 values. In the titration of ammonia with acetic acid in methanol, the first estimate may be in error by 10%; with practice this error can be made smaller. Even so, two or three successive calculations are likely to be required.

When the convergence of successive end point estimates is poor, an alternative method of calculation may be used. Instead of making a single first estimate of the end point, several estimates are made in such a way as to bracket the equivalence point. For each of these estimates a single four-point calculation is made, and the calculated end point is plotted *vs.* the estimated point. The correct end point is found on the plot as the point where the calculated and the estimated end points are equal. This method has the advantage that the convergence limit is approached from both directions.

Practical Example. Figure 4 shows the conductometric titration curve for 0.008N sodium azide with 0.1N hydrochloric acid in water. The titration data were obtained on a Shedlovsky bridge (*8*) with earphone detector. The temperature of the titration cell was constant to within 0.2° C. The cell constant was too large to obtain maximum accuracy in the resistance measurements (*5*), but the experimental points fell on a smooth curve with a mean deviation of 0.5%. In Figure 4, the ordinate is $(V + V_0)/V_0R$, where V is the volume of titrant, V_0 the initial volume of sodium azide, and R the measured resistance. The factor $(V + V_0)/V_0$ corrects for the dilution during the titration.

Table V. End Point Calculation for Titration of 0.008N Sodium Azide with 0.1N Hydrochloric Acid in Water

ϵ_1	ϵ_2	ϵ_3	ϵ_4	End Point, Ml.
0.7	0.9	1.3	1.11	19.90
0.85	0.89	1.2	1.1	19.99
0.6	0.93	1.2	1.1	19.75
0.6	0.915	1.3	1.1	19.97
			Av.	19.90 ± 0.08

A first estimate of 19.44 ml. for the end point was obtained by extrapolation of the nearly linear segments observed for $0 < V < 10$ and $V > 22$. A single calculation, using the four points 0.7, 0.9, 1.3, and 1.11 multiplied by 19.44 ml., led to an end point volume of 19.89 ml. A second calculation, using 19.89 ml. as the end point estimate, led to 19.90 ml. This end point was checked with three other sets of values of ϵ_1, ϵ_2, ϵ_3, and ϵ_4. The results are shown in Table V. The mean deviation of the four values was only 0.4%; all five calculations took less than 30 minutes.

This titration is comparable to that of sodium acetate because the K_A values of acetic and hydrazoic acids are nearly equal. The relatively large discrepancy between the first estimate and the final end point is not surprising. For similar ϵ-range selections, the tentative end point in the titration of sodium acetate occurred at $\epsilon = 0.974$, as shown in Table I. This is in good agreement with the observed ratio, 19.44/19.90 = 0.977. By the potentiometric method this titration would have been very difficult.

ACKNOWLEDGMENT

The author wishes to thank the Yad Chaim Weizmann for the award of a Chaim Weizmann Fellowship, and Dan Golomb for supplying the titration data for Figure 4.

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RECEIVED for review September 26, 1955. Accepted March 24, 1956.

Automatic Photometric Titrations

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A simple logarithmic attenuator circuit which makes possible the direct recording of absorbance from the output of a Beckman Model B spectrophotometer is described. When used in conjunction with a strip-chart recording potentiometer and a constant-delivery reagent system, the apparatus is well adapted to automatic photometric titrations. The application to automatic iodometric and acidimetric procedures is described.

AMONG several physicochemical methods available for locating titration end points automatically, measurement of light absorption has received surprisingly little attention. Several workers have suggested arrangements in which a large change in transmittance at the equivalence point could operate a trigger mechanism or be recorded as an indicator-instrument deflection (*2, 5, 7, 14, 15*). For many reactions, however, the equivalence point must be located by examination of an absorbance *vs.* volume curve (*4*). Heretofore, the only reported application of this technique to automatic titration has been that of

Malmstadt and others (*11, 12*), who performed their titrations in a Cary recording spectrophotometer. There would be considerable advantage to being able to use the more commonly available and much less expensive single-beam manual spectrophotometers, such as the Beckman Models B and DU, for automatic recording of titrations. This requires, however, some means of converting the output signal voltage of these instruments, which is linear with transmittance, to a logarithmic equivalent proportional to absorbance. The main portion of this paper is concerned with the description of a simple electronic converter which accomplishes this for the Beckman Model B spectrophotometer.

THEORY

Several electronic circuits which are available for logarithmic attenuation (*1, 6, 13*) might be modified to suit the requirements of the photometric titration. Of these, two appear to be particularly attractive: the circuit which Müller (*13*) employed in an early linear absorbance-scale colorimeter, because of its simplicity; and that proposed by Howard, Savant, and Neiswander (*6*), because of its stability and accuracy. The circuit of Howard, Savant, and Neiswander was logarithmic as low as 0.3-volt input signal, but it was relatively complex. Although it was proposed several years ago, Müller's circuit showed the greatest promise of easy adaptability to the Model B spectrophotometer, and a modification of it was developed accordingly.

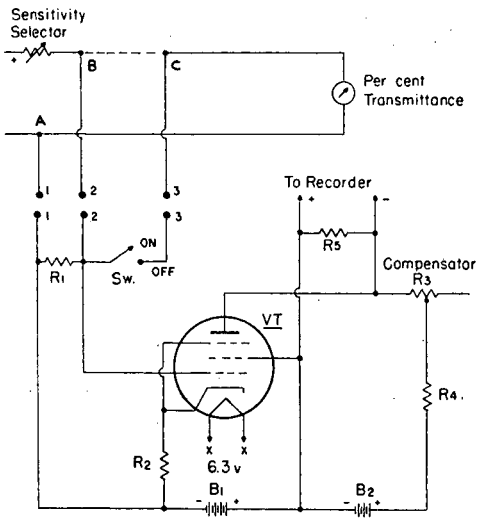

Figure 1. Logarithmic attenuator circuit

1, 2, 3. Leads entering spectrophotometer
A, B, C. Connections made within spectrophotometer circuit
R_1. 0.1-megohm precision type resistor
R_2. 2500-ohm precision type resistor
R_3. 100-kilo-ohm W. W. variable resistor
R_4. 1-kilo-ohm resistor, 1/4 watt
R_5. 0- to 100-ohm precision decade resistance box
VT. 6SK7-GT
B_1. 60-volt stabilized power supply
B_2. 1.5-volt Burgess 4FH battery
Sw. Switch

The vacuum tube selected for the critical logarithmic attenuation was the 6SK7-GT, the operational characteristics of which are described elsewhere (*16*). The important fact is that at plate operating potentials greater than 60 volts, the plate current does not follow the grid bias in a linear manner but more closely approximates the relationship

$$e^{-kI_b} = E + Z$$

where e is the base of the Naperian logarithms, I_b is the plate current, E is the grid potential, and k and Z are constants. Rewriting this equation in the logarithmic form, one obtains the relationship

$$I_b = k' \log \frac{1}{E + Z}$$

The actual absorbance relationship desired for photometric titrations follows the equation

$$A_s = \log \frac{100}{\%T}$$

Thus, by the selection of operating conditions for the 6SK7-GT where Z is small, one would expect to obtain the desired logarithmic attenuation of the spectrophotometer output signal. It is noted that the response of the 6SK7-GT comes to a finite value at $E = 0$ and one would expect some serious deviations from logarithmic attenuation at low input voltages. If the absorbance scale is to cover the range of 100 to 0% transmittance, the deviation would occur at high absorbances.

EXPERIMENTAL

Apparatus. The attenuator (Figure 1) was built into a metal cabinet 3 × 4 × 5 inches. The front cover of the Beckman Model B spectrophotometer was removed, the lead to the plus side of the transmittance meter was cut, and the necessary soldered connections, *A*, *B*, and *C*, Figure 1, were made to the attenuator circuit. When the cover was replaced, the leads for the attenuator were most conveniently brought out of the hole provided by the manufacturer for convenience in adjusting wavelength calibration.

When switch S_w is in the off position, the attenuator and recorder do not respond and the instrument can be used in the normal manner. Recorder sensitivity is determined by the setting of R_5 and zero adjustment made with R_3.

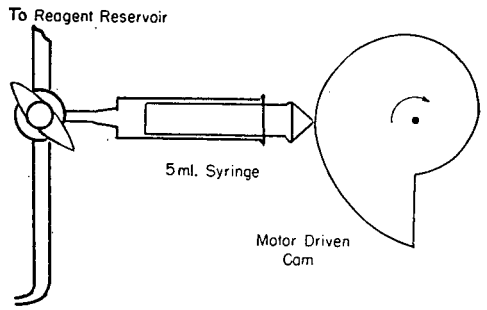

Figure 2. Reagent delivery system

The compensation control of the logarithmic attenuator was mounted externally so that zero adjustments could be made easily. A precision decade resistance box was used as the dropping resistor (R_5) to facilitate the use of the attachment with recording potentiometers of different ranges. If a fixed resistance is desired, a 100-ohm resistor is suitable for use with a 5-mv. range recorder. The use of a variable dropping resistor gives the added advantage that the attenuator can be adjusted to several different absorbance ranges when used with a fixed-range recorder. The operating plate and screen grid voltages were supplied by a commercially available stabilized power supply (Kepco Laboratories, Flushing, N. Y.) which could, however, be replaced by conventional B batteries. It was found that a more constant potential than could ordinarily be obtained with the power supply was needed for the attenuator compensation control; hence, a 1.5-volt dry cell was used for this function.

The actual recording of the absorbance during a photometric titration was made with a Weston recording potentiometer with a sensitivity of 5-mv. full-scale deflection. The chart speed was 2 inches per minute.

A cam-driven syringe buret was used to provide a constant flow of titrant (*8*). The assembly was similar to that used by Keily (*8*) for differential thermometric titrations and is shown schematically in Figure 2. The outside curvature of the cam was made to conform to the equation $\rho = k + a\theta$, so that when the cam was made to rotate at a constant speed, the volume displacement of the syringe would be linear with time. The cam used in this work conformed to the equation $\rho = 2.000$ inches $+ \frac{0.750}{\pi}$ inch. Thus, for each 0.360° angular displacement of the cam, a linear displacement of 0.0015 inch should occur in order to preserve reasonable uniformity of flow. In this work the steel cam was driven at 0.1 r.p.m. by a Bodine Type K-2 synchronous motor operating through a 10 to 1 worm gear reduction. The head of the piston of the syringe was fitted with a glass prism to provide point contact on the cam surface. The contact surface was lubricated slightly with stopcock grease and a small spring was attached to the piston to hold it securely in contact with the cam.

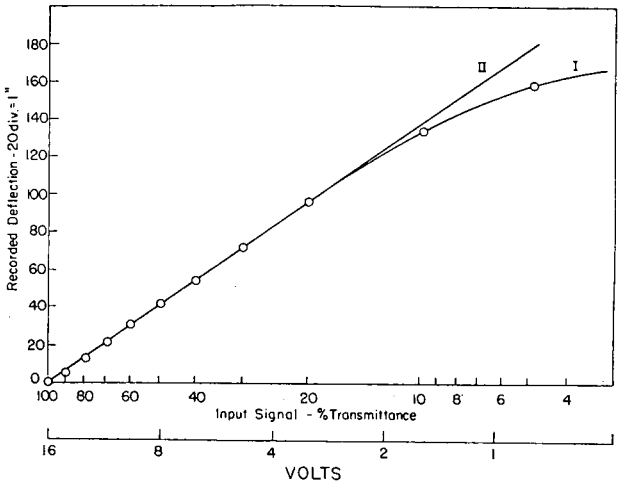

Figure 3. Test of attenuator response linearity

Abscissa represents input signal in volts from spectrophotometer; equivalent per cent transmittance readings on sensitivity "1" position also given

The volume delivery of the buret was calibrated by weighing aliquots; the maximum deviation from constant flow over 90% of the cam surface was 0.01 ml., indicating that the cam radius was accurate to 0.003 inch. When the buret was used in conjunction with the Weston recording potentiometer, the volume delivery was 0.2076 ml. per inch of chart. The cam buret could, of course, be replaced by other means of constant reagent addition, such as the screw-activated syringe delivery unit of Lingane (*10*).

It was found necessary to have the buret delivery tip drawn into a fine capillary and positioned as far as possible from the light path in the titration beaker. If the buret was allowed to empty near the light path, small erratic variations were produced in the absorbance record during the titration.

The photometric titrations were performed in the Beckman Model B spectrophotometer which had been equipped with the titration cell compartment previously described (*3*). To facilitate reproducibility of the beaker position in the light path, two glass arms were fused to opposite sides of the titration beaker. The beaker was placed in the light path so that the arms fitted into slotted wooden blocks which were a part of the beaker stand. A 100-ml. beaker was used in all of the present work. The volume of the solution in the beaker was adjusted to 80 to 85 ml., so that air was not introduced into the light path when stirring the solution during a titration.

When it was necessary to perform the titration in an atmosphere of nitrogen, the cell was covered with a thin sheet of polyethylene in which a small slit was cut for the entrance of the buret tip. After an initial purging for 5 minutes with prepurified nitrogen, further passage of gas was found unnecessary, because entrance of air was prevented by the polyethylene film.

Reagents. All the chemicals used in this work were of reagent grade and all solutions were prepared from deionized water of 1 to 2.5 × 10^6 ohms specific resistance. The nitrogen used for deaeration of the solutions was Air Reduction Co., prepurified grade.

Calibration of Logarithmic Attenuator. In order to ensure that the logarithmic attenuator produced an accurate signal conversion, a test was made over the 0 to 100% transmittance range. The results shown in Figure 3 indicated that serious deviations from logarithmic response occurred only when the input signal voltage was less than that corresponding to about 15% transmittance on the "1" scale (about 3.2 volts). The sensitivity selector (R_5) was therefore adjusted so that only the region above 20% transmittance appeared on the recorder scale.

Table I shows the response obtained with the logarithmic attenuator and the Weston recorder. The average deviation of the logarithmic attenuation from theoretical response (0.003 absorbance unit), while not suitable for precision photometric measurements, is satisfactory for photometric titrations. The attenuator alone gives a linear representation of absorbance over the range from zero to approximately 0.8 absorbance unit. When used in conjunction with the sensitivity range selector of the Model B spectrophotometer, the operating range becomes zero to 2.3 absorbance units in four steps. This range is adequate for most titrations; therefore, no attempt was made to extend the logarithmic response of the attenuator.

RESULTS

To test the response of the various components of the titration apparatus, several titration procedures were examined. The first was a scaled-down version of the conventional titration of iodine, generated from potassium iodate and acid, with sodium thiosulfate. This titration seemed particularly important because iodometric determinations are so widely applicable.

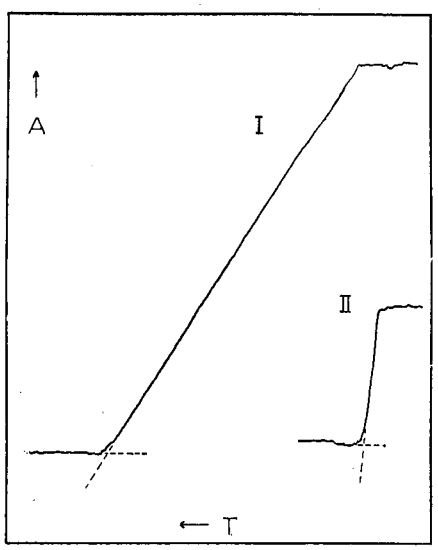

Figure 4. Titrations with 0.01*N* thiosulfate

I. 0.0001*M* iodate solution in presence of acid and excess iodide; λ = 450 mμ
II. 4.0-gram potassium iodide blank; λ = 365 mμ, maximum sensitivity

Solutions of iodate and thiosulfate were prepared as suggested by Kolthoff (*9*); the concentrations were such that they could be standardized on a macro scale to better than 1 p.p.t. Aliquots of these reagents were taken to prepare the dilute solutions necessary for the photometric titrations.

Table II shows the reproducibility of the titrations with

sodium thiosulfate. The standard deviation of replicates titrated with 0.01N thiosulfate was approximately 1%, which is consistent with the reliability of the automatic buret. The curves in Figure 4 indicate the type of curve obtained when titrating with 0.01N thiosulfate. The wave lengths were selected so that an absorbance change of about 0.5 unit occurred. For the blank estimation the wave length was selected so that a maximum change in absorbance resulted.

Table I. Logarithmic Attenuator Response

Transmittance, %	Input Signal, Volts	Response, Absorbance Unit		
		Recorder	Theoretical	Dev.
100	16.00	0.000	0.000	0.000
90	14.40	0.039	0.046	−0.007
80	12.80	0.092	0.097	−0.005
70	11.20	0.152	0.155	−0.003
60	9.60	0.223	0.222	+0.001
50	8.00	0.306	0.301	+0.005
40	6.40	0.401	0.398	+0.003
30	4.80	0.525	0.523	+0.002
20	3.20	0.699	0.699	0.000

Average deviation from theoretical response of logarithmic attenuator, 0.003 absorbance unit.

The increased variability of the titrations made with 0.0008N thiosulfate can be attributed partially to the slowness of the reaction at these extreme dilutions. The titration curves here did not show the usual abrupt changes in absorbance at the start or end of the reaction.

Table II. Automatic Photometric Titration of Microequivalent Quantities of Iodate

Equivalents × 10^6		Chart, Inches	Trials	Std. Dev., %	Error, %
Taken	Found[a]				
8.72	8.70	4.33	5	1.7	−0.34
4.36	4.2	2.23	5	1.1	−1.6
4 grams KI (blank)	0.40	0.19	5	13.0	..
10 grams KI (blank)	0.78	0.37	2	..	..
0.872	0.852[b]	6.87	3	8.4	−2.3
4 grams KI (blank)	0.30[b]	1.80	2	..	..

[a] Corrected for blank unless a blank determination.
[b] Titrated with 0.000809N sodium thiosulfate; others titrated with 0.01012N thiosulfate.

As an example of a neutralization procedure, the titration of chromate to dichromate with hydrochloric acid was studied. Chromate is the anion of a weak acid ($K_A = 3.2 \times 10^{-7}$) and the conversion may be followed spectrophotometrically in the region from 400 to 430 mμ. It was found desirable to remove carbon dioxide with nitrogen, but otherwise the performance of the titrations required no unusual arrangements. Titrations, particularly at high dilution, showed rounding at the equivalence point caused by partial dissociation of dichromate ion, but in every case extrapolation of the straight line portions of the titration plot could be made. As before, the solutions were standardized on a macro scale before dilution and titration. The reproducibility of the chromate titrations is shown in Table III; the titration curves are similar to those of the iodine-thiosulfate system and are not shown.

DISCUSSION

The application of the automatic equipment for routine analytical determinations appears to be limited principally by the need of a rapid reaction. Reaction velocity has been found to be an important factor even in the conventional iodine-thiosulfate titration when the concentration of thiosulfate titrant was less than 1×10^{-3} N. The requisite of a fast reaction is not peculiar to photometric titration, but is a factor which must be considered in all automatic titration methods. A second factor which may be a source of difficulty is the need for constant delivery rate of reagent. Malmstadt (*12*) alleviated this problem by the use of coulometrically generated titrant. The need for constant flow is particularly important when using the Cary recording spectrophotometer, because absorbance is recorded as a direct function of time. In the work described here the substitution of an X-Y type recorder for the X-T type Weston instrument could be easily made. In this case the volume delivery need not be constant if the delivery is directly coupled to one axis of the recording instrument. This type of system is used in the Precision-Dow Recordomatic Titrometer (*17*).

Table III. Automatic Photometric Titration of Chromate

Equivalents × 10^6		Chart, Inches[a]	Trials	Std. Dev., %	Error, %
Taken	Found				
50.20	50.8	4.69	5	1.0	+1.2
25.1	24.9	2.30	4	1.0	−0.7
5.02	4.80	4.42[b]	4	3.2	−4.4

[a] Chart equivalent, 0.2076 ml. per inch.
[b] Titrated with 0.00522N hydrochloric acid; others with 0.05220N hydrochloric acid.

In all of the titrations reported in this work, the addition of titrant produced an immediate decrease in the absorbance of the solution. Thus, it was possible to determine both the start and the equivalence point of the titration from the recorded plot. In titrations where the addition of titrant does not produce such a change, the start of the titration can be determined by always starting the buret at a clearly identifiable chart marking. Preliminary investigations indicate that this is a suitable method for permanganate oxidations.

ACKNOWLEDGMENT

The authors are indebted to the Atomic Energy Commission for partial support of this work.

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RECEIVED for review November 14, 1955. Accepted March 2, 1956.

Behavior of Acid-Base Indicators in Acetic Acid System

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Results of spectrophotometric and potentiometric studies on 13 indicators in anhydrous acetic acid suggest that the color changes which occur during titration are governed largely by the relative concentration of the base present and its conjugate acid. For strongly basic indicators such as ethyl red, acid colors can be elicited, furthermore, in acetic acid by neutral salts such as sodium perchlorate and sodium sulfate. The following indicators were studied: ethyl red, pinacyanol, 4-dimethylamino-4′-nitrostilbene, 2-nitro-9-(4′-dimethylaminobenzal)fluorene, 4-dimethylamino-4′-sulfamylazobenzene, quinaldine red, 4′-dimethylaminobenzalrhodanine, *m*-nitro-*N*,*N*-dimethylaniline, Brilliant Cresyl Blue, 1-naphtholbenzein, Nile Blue A, Sudan III, and Sudan IV. Their response was determined to the following buffer systems: ephedrine acetate–ephedrine perchlorate, sodium acetate–sodium perchlorate, sodium acetate–sodium sulfate, antipyrine acetate–antipyrine perchlorate, urea acetate–urea perchlorate, and perchloric acid, all in acetic acid.

DESPITE the voluminous number of publications related to the use of acetic acid and other nonaqueous solvents as titration media in analytical determinations, investigation of indicator behavior in these systems has been relatively limited. Experimental data are given here pertaining to the behavior of 13 indicators in acetic acid in the presence of a number of different cations and anions.

THEORY

Because the physical chemistry of color changes of indicators in nonaqueous solvents such as acetic acid is fundamentally different from that in the more familiar aqueous systems, it may be well to review very briefly the basic differences between the two systems.

In water, because of its high dielectric constant, all true salts can be considered to be both totally ionized and dissociated. For this reason the color of an indicator solution is determined primarily by the hydrogen ion concentration or the pH of the system. Thus, for indicators such as methyl orange, these relationships exist:

$$(H^+)_{H_2O} + I \rightleftarrows IH^+$$

and

$$\log \frac{I}{IH^+} = -\log H^+_{H_2O} - \log K_I = pH - pK_I \qquad (1)$$

where I represents the base form of the indicator; IH^+, the acid form; and K_I, the dissociation constant of acid IH^+.

For aqueous solutions containing a weak acid and its salt—e.g., benzoic acid—the following relationships also exist.

$$HBz + I \rightleftarrows IH^+ + Bz^-$$

The equilibrium constant for this reaction is

$$K = \frac{(IH^+)(Bz^-)}{(HBz)(I)} = \frac{K_I}{K_a} \qquad (2)$$

This equilibrium constant can be determined by determination of the ionization characteristic of the indicator and of the acid individually.

In solvents of low dielectric constant, such as acetic acid, the comparable equations are somewhat more complex because of the very strong tendencies of the ionic components to form ion pairs and more highly associated species. The strongest acids and strongest bases in these systems dissociate relatively little, even at very low concentrations.

The present studies suggest that the behavior of acid-base indicators in acetic acid is best represented by the general reaction

$$SHA + IHAc \rightleftarrows IHA + SHAc \qquad (3)$$

where S is the solvent or some other protophilic base present; SHA is the interaction product of a strong acid, HA, and S; IHAc is the acetate form of the indicator, exhibiting its base color; and IHA is the acid form of the indicator resulting from interaction with HA. Therefore

$$K = \frac{(IHA)(SHAc)}{(SHA)(IHAc)} \qquad (4)$$

This follows, in part, logically from relationships presented earlier (*7*):

$$K = \frac{K_a K_b}{K_{HAc} K_{ab}}$$

where K_a = dissociation constant of the acid
K_b = dissociation constant of the base
K_{HAc} = autoprotolytic constant of acetic acid
K_{ab} = dissociation constant of the resulting salt

Although both the base form of the indicator (IHAc) and the acid form (IHA) are written as products of acid-base reaction, the extent of proton transfer must be sufficiently different qualitatively to produce the observed color difference. Perchloric acid appears to be able to effect a substantial or a nearly complete transfer, whereas acetic acid probably forms only a hydrogen bond with the protophilic center. The proton transfer from a strong acid (HA) to the solvent or other protophilic base (S) to form an interaction product (SHA) is probably limited if S is an acetic acid molecule, but the transfer is nearly complete if S is, for example, an aliphatic amine. In the latter instance the over-all reaction can be written more simply as

$$M^+A^- + IHAc \rightleftarrows M^+Ac^- + IHA$$

and

$$K = \frac{(M^+Ac^-)(IHA)}{(M^+A^-)(IHAc)} \qquad (5)$$

where M^+A^- is a salt of a strong acid and a strong base, and M^+Ac^- is the corresponding acetate salt. A protonated ephedrine cation, for example, can play the role of M^+ in the above equation.

Because cations of this type are not basically different from elemental cations such as Na^+, K^+, and the like, the same equation may be expected to hold for these. An example of such a reaction is the interaction of ethyl red with sodium perchlorate, which is discussed below. In these cases, however, the over-all reactions do not involve proton transfer and there may be some question as to whether these should be considered as acid-base reactions.

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If concentrations are used in calculation of the equilibrium constant, the foregoing equations would be expected to be only approximate. It can be readily shown, for example, that

$$K = \frac{(K_{M^+Ac^-})\ (K_{IHA})}{(K_{M^+A^-})\ (K_{IHAc})} \qquad (6)$$

where

$$K_{M^+Ac^-} = \frac{(M^+)\ (Ac^-)}{(M^+Ac^-)}$$

$$K_{IHA} = \frac{(IH^+)\ (A^-)}{(IHA)}$$

$$K_{M^+A^-} = \frac{(M^+)\ (A^-)}{(M^+A^-)}$$

$$K_{IHAc} = \frac{(IH^+)\ (Ac^-)}{(IHAc)}$$

Because the values of the individual dissociation constants would be significantly affected by the presence of large concentrations of other ion pairs and ions in solution, K would be similarly influenced but probably not to the same extent.

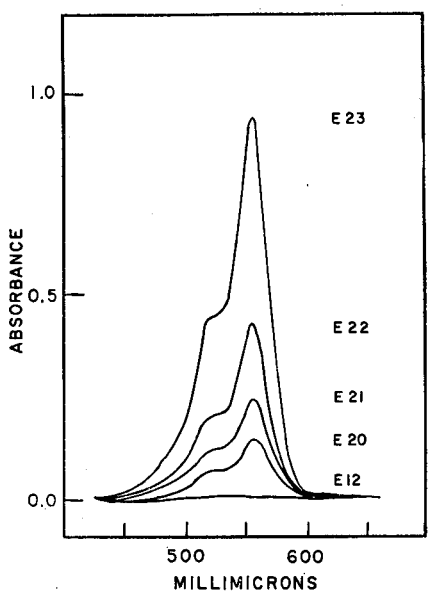


Figure 1. Absorption spectra of ethyl red

Ephedrine acetate–ephedrine perchlorate solutions in acetic acid

The difference in the apparent complexity of an indicator reaction in water from that in acetic acid is evident in comparing Equation 2 with Equation 6. In the latter case four dissociation constants must be known to permit a priori determination of the over-all equilibrium constant as compared to two for the former.

EXPERIMENTAL

Indicators. Ethyl red (Eastman Kodak No. 2155). Recrystallized from ethyl alcohol–ether mixture. Melting point, 150–152° C.

Pinacyanol (Eastman Kodak No. 622). Recrystallized as above. Melting point greater than 270° C.

4-Dimethylamino-4′-nitrostilbene. Synthesized according to the procedure of Pfeiffer (*8*). Melting point, 250–251° C.

2-Nitro-9-(4′-dimethylaminobenzal)fluorene. Prepared in a manner similar to above by condensing dimethylaminobenzaldehyde with 2-nitrofluorene. Melting point, 180–182° C.

4-Dimethylamino-4′-sulfamylazobenzene. Synthesized according to the procedure of Van Lente and Pope (*10*).

4′-Dimethylaminobenzalrhodanine. C.P. grade (Fisher Scientific), used directly.

m-Nitro-*N*,*N*-dimethylaniline (Eastman Kodak No. 1208). Recrystallized from ethyl alcohol. Melting point, 65° C.

Brilliant Cresyl Blue (Harleco). Recrystallized from petroleum ether.

Nile Blue A (Harleco). Recrystallized from petroleum ether. Melting point, 183° C.

1-Naphtholbenzein (Eastman Kodak No. 924). Recrystallized from ethyl alcohol.

Sudan III (National Aniline). Recrystallized from ethyl alcohol–water mixture. Melting point, 194° C.

Sudan IV (National Aniline). Recrystallized as above. Melting point, 184° C.

Solvents and Solutions. Acetic acid, used in preparing all solutions studied, was purified by the method of Eichelberger and La Mer (*1*). The product showed a water content (Karl Fischer) of less than 0.01%. For the relatively nonacidic ranges covered in most of this investigation, a small amount of water would be expected ordinarily to have relatively minor influences on the results.

All perchlorate solutions were prepared by treating the corresponding acetate solutions with dilute perchloric acid solution. The latter, usually 0.0555*M*, was made by diluting an approximately 1*M* solution prepared according to the procedure of Fritz (*2*). The sulfuric acid and sulfate solutions were prepared from anhydrous sulfuric acid, sodium acetate, and purified acetic acid.

Spectrophotometric Determination of Exchange Constant. A salt solution of the desired composition was prepared by mixing the proper volumes of the selected base and its perchlorate solution in a manner similar to that described by Hall and Meyer (*3*). To 9 ml. of the resulting buffer solution, 1 ml. of indicator solution (approximately 0.02%) was added. The absorption spectrum of this solution was then recorded in the region from 325 to 800 mμ by means of a Cary recording spectrophotometer, in which 1-cm. Corex cells were used.

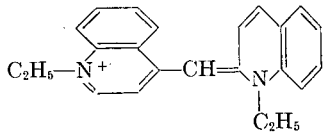
In order to obtain the pure spectra of the indicators in their acidic and basic forms, recordings were also made in the presence of excess perchloric acid and a base sufficiently strong so that no spectral change occurred when a stronger base was employed.

From the spectral data the ionization ratio of the indicator in solution was determined and used to calculate the equilibrium constant defined by Equation 4, except for the K values in Table VIII.

Determination of Potentiometric Acidity. The potentiometric response of all final solutions toward a glass electrode was determined. A Beckman glass electrode No. 290 and a Beckman calomel electrode No. 270–271 were used as such, except that the electrolyte in the calomel electrode was replaced with a 0.02*M* lithium chloride solution in acetic acid. To correct for junction potentials and any asymmetry effect, all readings of electromotive force in the following tables were taken relative to the potential observed for a 0.100*M* sodium salicylate solution in acetic acid. Measurements were made with a Beckman Model G pH meter.

BEHAVIORS OF INDIVIDUAL INDICATORS

Ethyl Red.



Ethyl red, an isocyanine dye that has been used in photography, is a one-color indicator in acetic acid. The alkaline form is red and the acid form is colorless. As far as is known, the indicator has never been used in acid-base titrimetry. It is the most basic of the indicators in this study and is more basic than any of the indicators that have been used previously in acetic acid.

Figure 1 shows the influence of ephedrine acetate, which had been neutralized to a varying extent with perchloric acid, on the spectral property of the indicator. The compositions of the ephedrine acetate–ephedrine perchlorate solutions shown in the

figure are given in Table I, where the corresponding equilibrium constants are also listed for the following reaction:

$$\text{Eph.H}^+\ldots\text{ClO}_4^- + \text{ER.HAc} \overset{K}{\rightleftarrows} \text{Eph.H}^+\text{Ac}^- + \text{ER.HClO}_4$$

From both the figure and the table it is apparent that the proton transfer in the acetate form of the indicator must be much less than with the perchlorate. Although no direct evidence is available in this instance, it seems probable that the reaction involves largely an exchange of anions and that the protons on the ephedrine and the indicator remain essentially on the cation throughout the reaction. The sizable value of the equilibrium constant (about 10) suggests that the indicator acetate is an effectively stronger base than ephedrine acetate in this system.

Behavior of the same indicator in the sodium acetate–sodium perchlorate system is also shown in Table I. The equilibrium constant for the reaction

$$\text{ER.HAc} + \text{NaClO}_4 \overset{K}{\rightleftarrows} \text{ER.HClO}_4 + \text{NaAc}$$

is roughly four times as large as in the preceding case and is in line with the known lower dissociation of the metal acetate.

Table I. Interaction between Ethyl Red and Several Buffer Systems in Acetic Acid

Solution	Molar Conen. Base	Molar Conen. Salt	E.M.F., Mv.	Absorbance, 558 mμ	$\frac{IH^+}{I}$	K
	Ephedrine acetate	Ephedrine perchlorate				
E 23 (I)	0.0500	0.0000	..	0.94	...	...
E 22	0.0444	0.0056	− 6	0.43	1.19	10
E 21	0.0389	0.0111	− 1	0.24	2.91	10
E 20	0.0333	0.0167	4	0.14	5.71	11
E 12 (IH$^+$)	0.0000	0.0500	..	0.00	...	...
	Sodium acetate	Sodium perchlorate				
N 1 (I)	0.0328	0.0000	..	1.35	...	...
N 2	0.0322	0.0006	18	0.80	0.687	37
N 3	0.0317	0.0011	20	0.56	1.41	41
N 4	0.0306	0.0022	22	0.33	3.09	43
N 5	0.0285	0.0043	24	0.17	6.94	46
N 6	0.0243	0.0085	27	0.07	18.3	52
N 7 (IH$^+$)	0.0115	0.0213	..	0.00	...	...
	Sodium acetate	Sodium sulfate				
N 1 (I)	0.0976	0.0000	...	1.22	...	...
N 2	0.0508	0.0017	35	1.05	0.162	4.8
N 3	0.0473	0.0035	39	0.95	0.284	3.8
N 4	0.0403	0.0070	42	0.77	0.585	3.4
N 6	0.0746	0.0061	38	0.98	0.245	3.0
N 7	0.0429	0.0061	43	0.88	0.386	2.7
N 8	0.0312	0.0061	46	0.73	0.671	3.4
N 9	0.0095	0.0061	51	0.32	2.81	4.4
N 10 (IH$^+$)[a]	...	...	..	0.00	...	...
	Antipyrine acetate	Antipyrine perchlorate				
H 2300 (I)	0.0500	0.00000	..	1.13	...	...
H 2202	0.0499	0.00010	25	0.60	0.883	440
H 2204	0.0498	0.00020	27	0.41	1.76	440
H 2206	0.0497	0.00030	28	0.28	3.04	500
H 2208	0.0496	0.00040	30	0.21	4.38	540
H 2210	0.0494	0.00060	31	0.17	5.65	470
H 1200 (IH$^+$)	0.0000	0.05000	..	0.00	...	...

[a] 0.0783M solution of H_2SO_4.

Because in this instance one of the cations is not protonated, the over-all reaction must involve straight anion exchange. For this system, the "acid" responsible for producing the color change is sodium perchlorate, a substance which in water does not exhibit the slightest acidic behavior.

The effect of a different anion on the interchange reaction is shown by the data for an ethyl red–sodium acetate–sodium sulfate system (Table I). A fairly reproducible constant can be calculated on the assumption that the reaction involves an exchange of an acetate ion on the indicator with one $NaSO_4^-$ ion of sodium sulfate. Although this ion appears to be as effective electronically as the perchlorate ion in producing the color change, it does not shift the reaction as much to the right as the perchlorate. This is probably due to the extremely low dissociative tendency of the normal sulfate salt in this system, as was recently shown by Higuchi and Rehm (7). Sodium perchlorate thus acts effectively more acidic than sodium sulfate in this system.

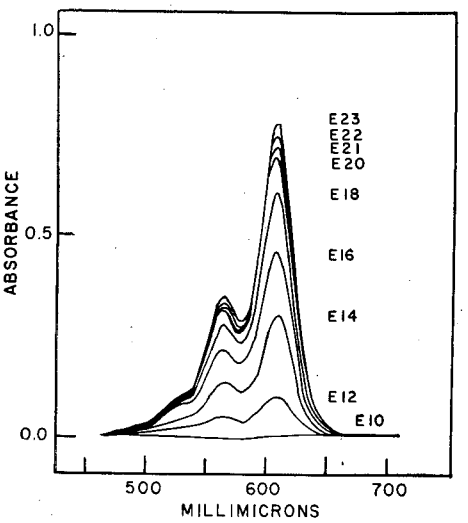
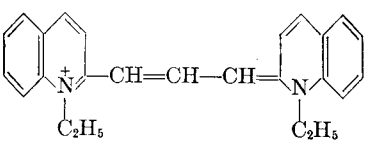


Figure 2. Absorption spectra of pinacyanol

Ephedrine acetate–ephedrine perchlorate solutions in acetic acid

Data for ethyl red in antipyrine perchlorate–antipyrine acetate system are also presented in Table I. Because the base present is weak, the equilibrium is largely to the right. Unlike the ephedrine system, the transfer of a proton to antipyrine from the solvent is probably limited.

Pinacyanol.

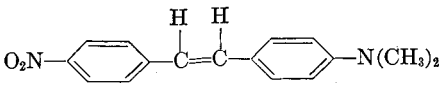


Pinacyanol is also an isocyanine dye that has been used in photography; it is available commercially as the iodide salt. It, too, is a one-color indicator—blue in the alkaline form and colorless in the acid form. As far as is known, this indicator has not been used for titrimetric purposes.

The absorption spectra of this indicator in the presence of several ephedrine perchlorate–ephedrine acetate buffer solutions in acetic acid are shown in Figure 2; the equilibrium constants for this system are given in Table II. From the weighted average value of 0.23 it is evident that the effective basicity of pinacyanol is significantly less than that of ethyl red.

Corresponding data for the sodium acetate–sodium perchlorate system and for the antipyrine acetate–antipyrine perchlorate system are also shown in Table II. The equilibrium data indicate that the interchange constant is approximately 1.6 for the sodium system and 13 for the antipyrine system.

4-Dimethylamino-4′-nitrostilbene.



4-Dimethylamino-4′-nitrostilbene is a one-color indicator, being yellow and colorless in the alkaline and acid forms, respectively. Addition of acids apparently interferes with its resonance, and the indicator solution becomes colorless. As far as is known, this is its first reported use in acetic acid titrimetry.

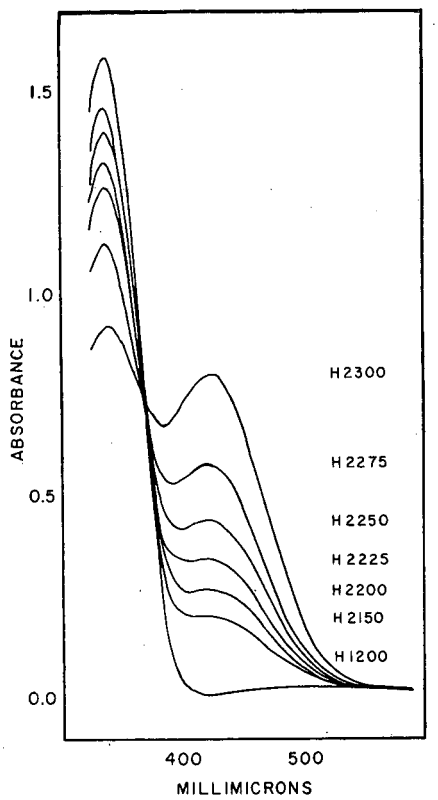
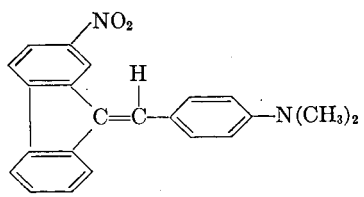


Figure 3. Absorption spectra of 4-dimethylamino-4′-nitrostilbene

Antipyrine acetate–antipyrine perchlorate solutions in acetic acid

The results of spectrophotometric determinations on 4-dimethylamino-4′-nitrostilbene for the antipyrine system are shown in Table III and Figure 3. From the equilibrium constant of approximately 15, it appears that this indicator is roughly comparable to pinacyanol in its strength.

2-Nitro-9-(4′-dimethylaminobenzal)fluorene.



This indicator can be considered as a stilbene-type indicator. It has not been reported previously in the literature. In acetic acid it undergoes a one-color change, from yellow to colorless in alkaline to acid media.

The absorption spectra of this indicator in the presence of antipyrine buffers in acetic acid are shown in Figure 4. The equilibrium constants calculated for the system are given in Table III An estimated value of 7 for the interchange constant suggests that this indicator is somewhat less basic than the preceding analog.

Table II. Interaction between Pinacyanol and Several Buffer Systems in Acetic Acid

Solution	Molar Concn. Base	Molar Concn. Salt	E.M.F., Mv.	Absorbance, 608 mμ	$\frac{IH^+}{I}$	K
	Ephedrine acetate	Ephedrine perchlorate				
E 23 (I)	0.0500	0.0000	...	0.78	...	...
E 22	0.0444	0.0056	−8	0.74	0.054	0.43
E 21	0.0389	0.0111	−3	0.72	0.083	0.29
E 20	0.0333	0.0167	3	0.70	0.114	0.23
E 18	0.0222	0.0278	14	0.61	0.279	0.22
E 16	0.0111	0.0389	31	0.46	0.696	0.20
E 14	0.0056	0.0444	44	0.30	1.60	0.20
E 12	0.0000	0.0500	75	0.10	6.80	...
E 10 (IH^+)[a]	...	...	...	0.00	...	...
	Sodium acetate	Sodium perchlorate				
N 23 (I)	0.0500	0.0000	...	1.81	...	...
N 22	0.0444	0.0056	20	1.50	0.210	1.68
N 21	0.0389	0.0011	27	1.24	0.467	1.64
N 20	0.0333	0.0167	36	1.02	0.790	1.58
N 18	0.0222	0.0278	53	0.61	2.04	1.53
N 16	0.0111	0.0389	77	0.29	5.63	1.61
N 14	0.0056	0.0444	94	0.13	15.3	1.93
N 12 (IH^+)	0.0000	0.0500	...	0.02	...	...
	Antipyrine acetate	Antipyrine perchlorate				
H 23 (I)	0.5000	0.0000	...	0.95	...	...
H 22	0.0444	0.0056	54	0.37	1.57	12.4
H 21	0.0389	0.0111	70	0.20	3.57	13.2
H 20	0.0333	0.0167	82	0.12	6.92	13.8
H 18	0.0222	0.0278	100	0.05	18.0	14.4
H 12 (IH^+)	0.0000	0.0500	...	0.00	...	...

[a] 0.0500M solution of $HClO_4$.

Table III. Interaction between Several Indicators and Antipyrine Acetate–Antipyrine Perchlorate System in Acetic Acid

Solution	Molar Concn. Antipyrine acetate	Molar Concn. Antipyrine perchlorate	E.M.F., Mv.	Absorbance	$\frac{IH^+}{I}$	K
4-Dimethylamino-4′-nitrostilbene				425 mμ		
H 2300 (I)	0.0500	0.0000	..	0.80	...	..
H 2275	0.0486	0.0014	38	0.58	0.380	13.2
H 2250	0.0472	0.0028	45	0.44	0.818	13.8
H 2225	0.0458	0.0042	50	0.35	1.29	14.0
H 2200	0.0444	0.0056	54	0.27	1.96	15.5
H 2150	0.0417	0.0083	60	0.20	3.00	15.1
H 1200 (IH^+)	0.0000	0.0500	..	0.00	...	..
2-Nitro-9-(4′-dimethylaminobenzal)fluorene				420 mμ		
H 2300 (I)	0.0500	0.0000	..	0.58	...	..
H 2250	0.0472	0.0028	47	0.41	0.415	7.0
H 2225	0.0458	0.0042	52	0.36	0.612	6.8
H 2200	0.0444	0.0056	57	0.30	0.934	7.4
H 2150	0.0417	0.0083	62	0.23	1.52	7.6
H 2100	0.0389	0.0111	70	0.16	2.66	9.3
H 1200 (IH^+)	0.0000	0.0500	..	0.00	...	..
4-Dimethylamino-4′-sulfamylazobenzene				510 mμ		
H 230 (I)	0.0500	0.0000	..	0.23	...	..
H 225	0.0472	0.0028	49	0.57	0.347	5.8
H 220	0.0444	0.0056	60	0.83	0.833	6.6
H 215	0.0417	0.0083	65	0.97	1.28	6.4
H 210	0.0389	0.0111	72	1.10	1.93	6.8
H 205	0.0361	0.0139	77	1.19	2.66	6.9
H 200	0.0333	0.0167	82	1.29	4.08	8.1
H 120 (IH^+)	0.0000	0.0500	..	1.55	...	..

4-Dimethylamino-4′-sulfamylazobenzene.

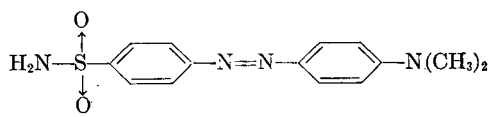


Table IV. Interaction between Quinaldine Red and Several Buffer Systems in Acetic Acid

Solution	Molar Concn. Base	Molar Concn. Salt	E.M.F., Mv.	Absorbance	$\frac{IH^+}{I}$	K
	Ephedrine acetate	Ephedrine perchlorate		525 mμ		
E 1 (I)	0.0400	0.0000	..	1.134	...	...
E 2	0.00200	0.0250	72	0.849	0.336	2.7×10^{-2}
E 3	0.00100	0.0250	91	0.689	0.646	2.6×10^{-2}
E 4	0.00050	0.0250	104	0.466	1.43	2.9×10^{-2}
E 5	0.00020	0.0250	136	0.223	4.09	3.3×10^{-2}
E 6	0.00040	0.0250	116	0.440	1.58	2.5×10^{-2}
E 7	0.00050	0.0100	96	0.767	0.478	2.4×10^{-2}
E 8 (IH^+)[a]	...	...	..	0.000	...	...
	Sodium acetate	Sodium perchlorate		525 mμ		
N 1 (I)	0.05000	0.0000	..	0.915	...	...
N 2	0.01000	0.0500	80	0.531	0.723	0.14×10^{-2}
N 3	0.00600	0.0500	92	0.410	1.23	0.15×10^{-2}
N 4	0.00300	0.0500	112	0.260	2.52	0.15×10^{-2}
N 5	0.00200	0.0500	121	0.205	3.46	0.14×10^{-2}
N 6	0.00100	0.0500	136	0.120	6.63	0.13×10^{-2}
N 7	0.00200	0.0250	112	0.325	1.82	0.15×10^{-2}
N 8	0.00200	0.0100	98	0.556	0.646	0.13×10^{-2}
N 9 (IH^+)[b]	...	...	..	0.000	...	...
	Antipyrine acetate	Antipyrine perchlorate		530 mμ		
H 23 (I)	0.0500	0.0000	..	1.11	...	...
H 22	0.0444	0.0056	62	0.96	0.156	1.3×10^{-2}
H 21	0.0389	0.0111	75	0.83	0.337	1.2×10^{-2}
H 20	0.0333	0.0167	84	0.70	0.586	1.2×10^{-2}
H 18	0.0222	0.0278	104	0.43	1.58	1.3×10^{-2}
H 16	0.0111	0.0389	125	0.19	4.84	1.4×10^{-2}
H 14	0.0056	0.0444	149	0.09	11.3	1.4×10^{-2}
H 12 (IH^+)	0.0000	0.0500	..	0.00	...	...

[a] 0.0522*M* solution of $HClO_4$.
[b] 0.0529*M* solution of $HClO_4$.

4-Dimethylamino-4′-sulfamylazobenzene, a typical azo dye, undergoes a two-color indicator change in acetic acid from yellow in the alkaline to red in the acid form. This indicator is slightly more basic and fluorescent than the parent compound, dimethylaminoazobenzene.

The absorption spectra for this indicator, shown in Figure 5, exhibit a strong acid band at 510 mμ. These curves were obtained in acetic acid in the presence of antipyrine buffers; they were used to compute the data for this indicator listed in Table III, where an exchange constant of approximately 7 is indicated for the antipyrine system.

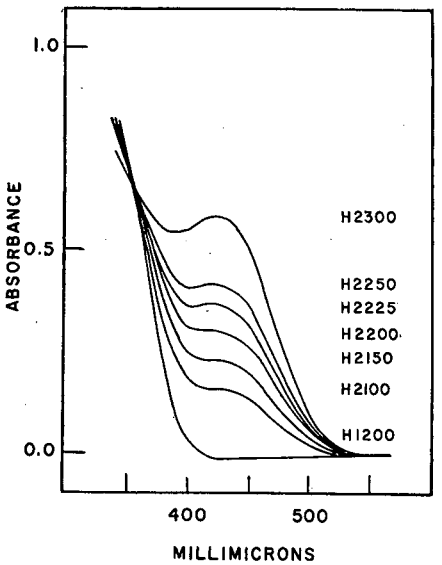


Figure 4. Absorption spectra of 2-nitro-9-(4′-dimethylaminobenzal)-fluorene

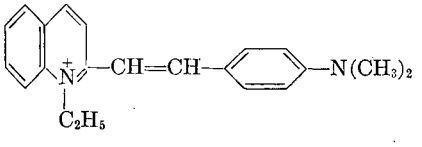
Antipyrine acetate–antipyrine perchlorate solutions in acetic acid

Table V. Interaction between Indicators and Antipyrine Acetate–Antipyrine Perchlorate System in Acetic Acid

Solution	Molar Concn. Antipyrine acetate	Molar Concn. Antipyrine perchlorate	E.M.F., Mv.	Absorbance	$\frac{IH^+}{I}$	K
4′-Dimethylaminobenzalrhodanine				467 mμ		
H 230 (I)	0.0500	0.0000	..	1.33	...	..
H 180	0.0222	0.0278	95	0.93	0.430	0.34
H 175	0.0194	0.0306	102	0.87	0.529	0.34
H 170	0.0167	0.0333	108	0.76	0.750	0.37
H 165	0.0139	0.0361	117	0.67	0.985	0.38
H 160	0.0111	0.0389	123	0.55	1.42	0.40
H 150	0.0083	0.0417	131	0.44	2.02	0.41
H 140	0.0056	0.0444	142	0.30	3.43	0.43
H 120 (IH^+)	0.0000	0.0500	..	0.00	...	..
m-Nitro-*N*,*N*-dimethylaniline				396 mμ		
H 23 (I)	0.0500	0.0000	..	0.59	...	..
H 22	0.0444	0.0056	59	0.54	0.093	0.74
H 21	0.0389	0.0111	75	0.51	0.157	0.55
H 20	0.0333	0.0167	83	0.49	0.204	0.41
H 18	0.0222	0.0278	102	0.38	0.553	0.44
H 16	0.0111	0.0389	125	0.24	1.46	0.42
H 14	0.0056	0.0444	138	0.15	2.93	0.37
H 12	0.0000	0.0500	160	0.07	7.43	..
H 10 (IH^+)[a]	...	...	..	0.00	...	..

[a] 0.0500*M* solution of $HClO_4$.

Quinaldine Red.



Quinaldine red, a stilbene derivative, has been used to a limited extent in acetic acid. Higuchi and Concha (*4*) have shown that it is a considerably more basic indicator in this solvent than the more commonly used crystal violet. Moreover, it is a simpler indicator to use because its color change is from deep red in basic solution to colorless in acid. Although the indicator shows some instability on standing in acetic acid solutions, this does not seriously detract from its utility.

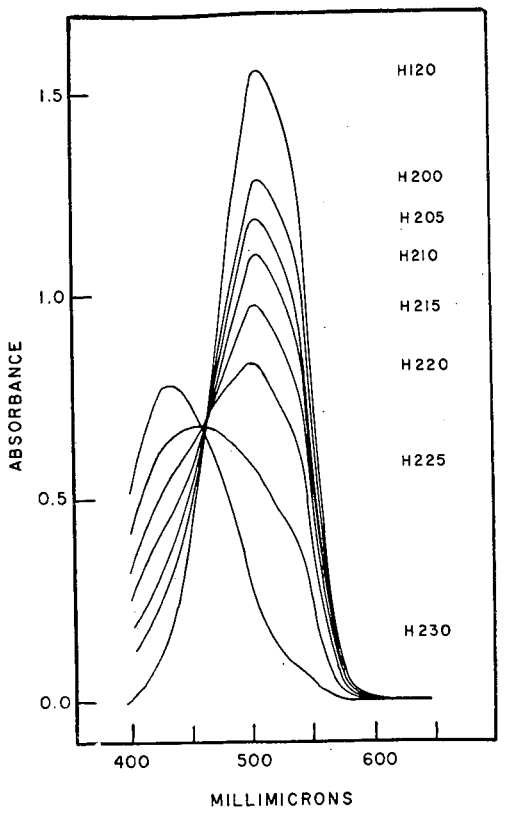
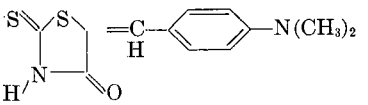


Figure 5. Absorption spectra of 4-dimethylamino-4′-sulfamylazobenzene

Antipyrine acetate–antipyrine perchlorate solutions in acetic acid

The absorption spectra and general data for the ephedrine acetate–ephedrine perchlorate system are shown in Figure 6 and Table IV. Because the equilibrium constant for the interchange reaction in this system is so low, the estimated value of roughly 0.027 is only approximate. The corresponding data for sodium acetate–sodium perchlorate and antipyrine acetate–antipyrine perchlorate are also given in Table IV. The indicated constants of 0.14 and 1.3, respectively, appear to be reasonable.

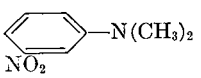
4′-Dimethylaminobenzalrhodanine.



4′-Dimethylaminobenzalrhodanine, like the other stilbene indicators, undergoes a one-color transformation in acetic acid titrations. The change is fairly sharp, from yellow in the alkaline to colorless in the acid form. Addition of acid suppresses the resonance possibilities in the dye, resulting in a colorless indicator solution.

The results of the spectrophotometric measurements for this indicator in an acetic acid solution of antipyrine acetate–antipyrine perchlorate are shown in Table V; the data indicate that the basicity is somewhat less than that of quinaldine red. Figure 7 shows the absorption spectra from which the data were obtained.

***m*-Nitro-*N*,*N*-dimethylaniline.**



m-Nitro-*N*,*N*-dimethylaniline is one of the simple basic indicators upon which the study of Hall and Meyer (*3*) was based. This indicator was included to permit comparison with studies of earlier workers. The compound acts as a one-color indicator in

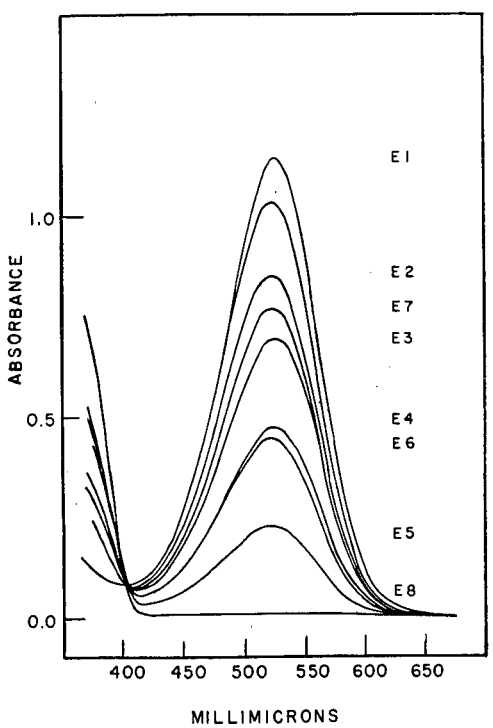


Figure 6. Absorption spectra of quinaldine red

Ephedrine acetate–ephedrine perchlorate solutions in acetic acid

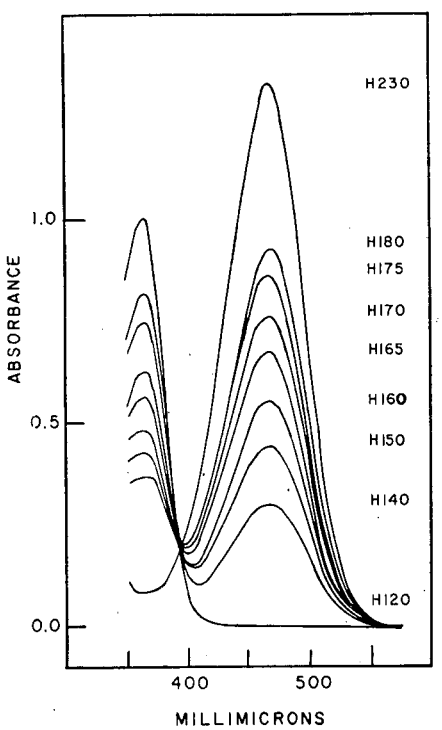


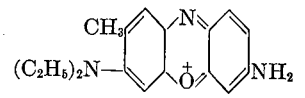
Figure 7. Absorption spectra of 4′-dimethylaminobenzalrhodanine

Antipyrine acetate–antipyrine perchlorate solutions in acetic acid

acetic acid, changing from yellow in basic to colorless in acidic solutions. Visually, the color transition appears to be very gradual.

The spectrophotometric results for this indicator in an acetic acid solution of antipyrine acetate–antipyrine perchlorate are shown in Table V, and the absorption spectra from which these data were obtained are given in Figure 8. The data indicate that the basicity is similar to that of the rhodanine derivative.

Brilliant Cresyl Blue.



Brilliant Cresyl Blue is an oxazine dye, and is available as the chloride salt. In working concentration it appears as a one-color indicator in acetic acid. The alkaline form of the indicator is blue, while the acid form exhibits a very faint pink tinge, the color transition being sharp. If the concentration of the indicator is increased, it behaves as a two-color indicator, but the end point becomes obscured.

The absorption spectra and results of spectrophotometric determinations of this indicator in acetic acid solutions of antipyrine acetate–antipyrine perchlorate are shown in Figure 9 and Table VI, respectively. Because of the extremely low interchange constant of this system, precise determination of the constant is difficult. It would appear, nevertheless, that the data are at least qualitatively correct. Results of a similar study in a more acidic medium are also shown in Table VI. Because urea is such a weak base, its salt would be expected to undergo solvolysis to a significant degree. The data, especially in the presence of preponderant concentrations of urea acetate, however, conform to the normal behavior.

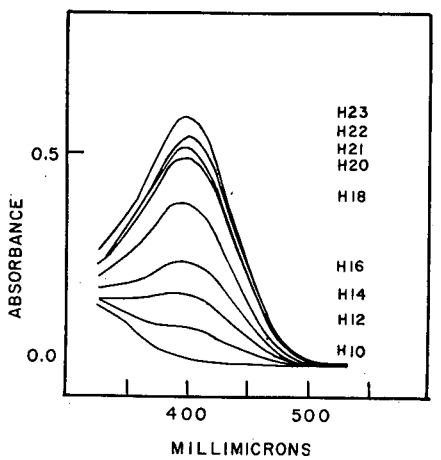
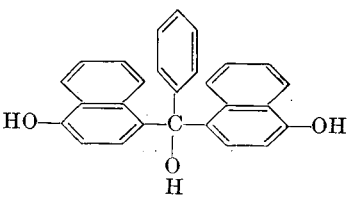


Figure 8. Absorption spectra of *m*-nitro-*N*,*N*-dimethylaniline

Antipyrine acetate–antipyrine perchlorate solutions in acetic acid

1-Naphtholbenzein.



1-Naphtholbenzein is a triaryl carbinol dye which, in acetic acid, undergoes a two-color change. It is yellow in the alkaline form and green in the acid form, and the color transition is sharp. It has been used by Higuchi and Concha (*4*, *5*) with quinaldine red in the differentiating titration of certain anionic species in acetic acid.

The absorption spectra and general data for this indicator in the urea acetate–urea perchlorate system are shown in Figure 10

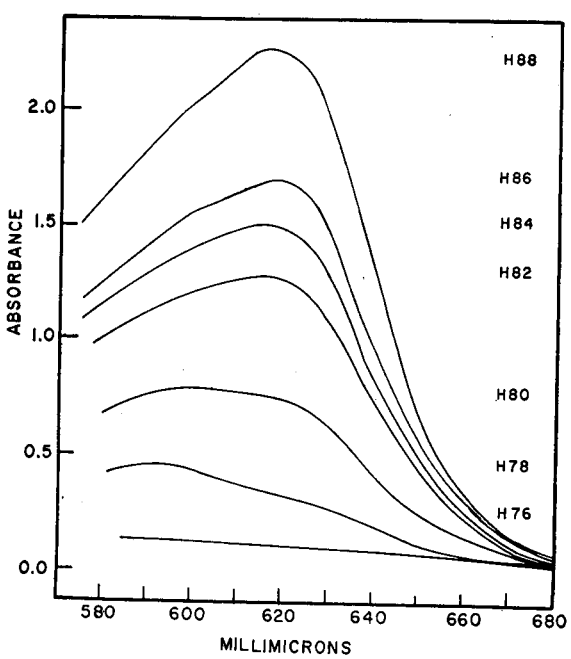


Figure 9. Absorption spectra of Brilliant Cresyl Blue

Antipyrine acetate–antipyrine perchlorate solutions in acetic acid

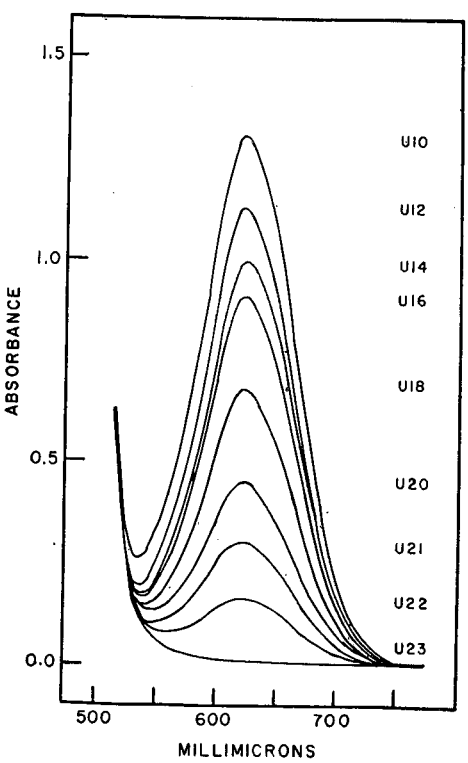


Figure 10. Absorption spectra of 1-naphtholbenzein

Urea acetate–urea perchlorate solutions in acetic acid

Table VI. Interaction between Brilliant Cresyl Blue and Buffer Systems in Acetic Acid

Solution	Molar Concn. Base	Molar Concn. Salt	E.M.F., Mv.	Absorbance, 617 mμ	$\frac{IH^+}{I}$	K
	Antipyrine acetate	Antipyrine perchlorate				
H 88 (I)	0.0500	0.0000	..	2.28	...	...
H 86	0.0020	0.0500	186	1.71	0.362	1.5×10^{-2}
H 85	0.0015	0.0500	199	1.46	0.607	1.8×10^{-2}
H 82	0.0010	0.0500	210	1.29	0.847	1.7×10^{-2}
H 81	0.0008	0.0500	223	1.0	1.41	2.2×10^{-2}
H 79	0.0005	0.0500	236	0.76	2.38	2.4×10^{-2}
H 80	0.0002	0.0250	244	0.77	2.35	1.9×10^{-2}
H 76 (IH^+)[a]	...	...	..	0.12	...	...
	Urea acetate	Urea perchlorate				
U 23 (I)	0.0500	0.0000	..	0.81	...	...
U 21	0.0389	0.0111	223	0.55	0.565	1.9×10^{-2}
U 20	0.0333	0.0167	231	0.48	0.847	1.7×10^{-2}
U 18	0.0222	0.0278	248	0.33	2.00	1.6×10^{-2}
U 16	0.0111	0.0389	265	0.24	3.80	1.1×10^{-2}
U 14	0.0056	0.0444	278	0.15	11.0	1.4×10^{-2}
U 12	0.0000	0.0500	299	0.13	17.0	...
U 10 (IH^+)[b]	...	...	..	0.09	...	...

[a] 0.0522*M* solution of $HClO_4$
[b] 0.0548*M* solution of $HClO_4$.

Table VII. Interaction between Indicators and Urea Acetate–Urea Perchlorate System in Acetic Acid

Solution	Molar Concn. Urea acetate	Molar Concn. Urea perchlorate	E.M.F., Mv.	Absorbance	$\frac{IH^+}{I}$	K
1-Naphtholbenzein				623 mμ		
U 23 (I)	0.0500	0.0000	..	0.00	...	..
U 22	0.0444	0.0056	208	0.16	0.147	1.17
U 21	0.0389	0.0111	221	0.30	0.316	1.11
U 20	0.0333	0.0167	230	0.45	0.564	1.13
U 18	0.0222	0.0278	249	0.68	1.19	0.96
U 16	0.0111	0.0389	263	0.90	2.57	0.74
U 14	0.0056	0.0444	280	1.00	4.00	0.50
U 12	0.0000	0.0500	292	1.12	...	..
U 10 (IH^+)[a]	...	...	..	1.25	...	..
Nile Blue A				630 mμ		
U 23 (I)	0.0500	0.0000	..	1.59	...	..
U 21	0.0389	0.0111	222	1.39	0.149	0.52
U 20	0.0333	0.0167	232	1.30	0.232	0.47
U 18	0.0222	0.0278	246	1.04	0.55	0.44
U 16	0.0111	0.0389	264	0.69	1.40	0.40
U 14	0.0056	0.0444	279	0.42	3.16	0.40
U 12	0.0000	0.0500	302	0.25	6.70	..
U 10 (IH^+)[a]	...	...	..	0.05	...	..

[a] 0.0500*M* solution of $HClO_4$.

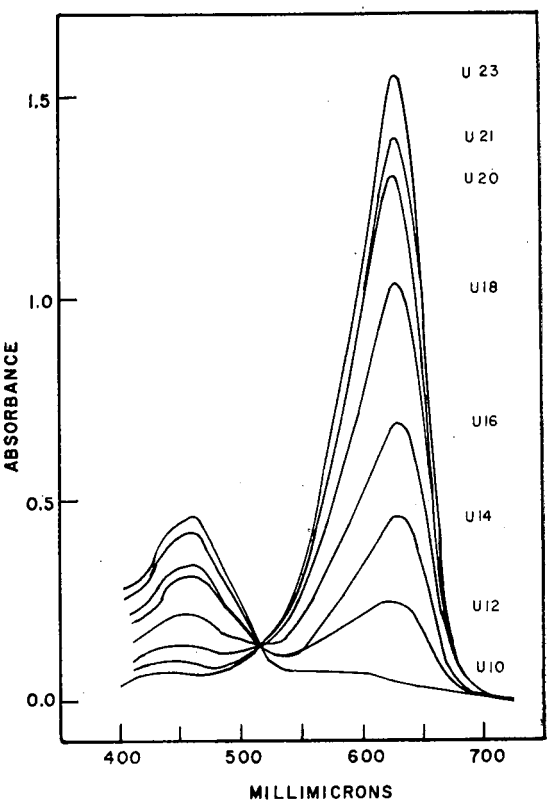
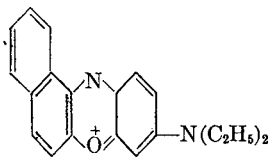


Figure 11. Absorption spectra of Nile Blue A
Urea acetate–urea perchlorate solutions in acetic acid

and Table VII, respectively. The evaluated equilibrium constant for this system is approximately 0.98, showing that this indicator exhibits an apparent basicity in acetic acid comparable to that of urea.

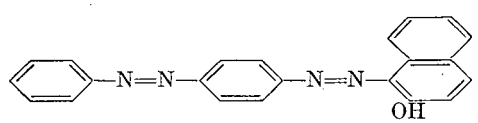
Nile Blue A.



Nile Blue A is a member of the oxazine series of dyes and is available commercially as the bisulfate salt. In very dilute solutions in acetic acid it undergoes an apparent one-color transition from blue in the alkaline to essentially colorless in the acid form. More concentrated solutions of the indicator exhibit a two-color transition from blue to light yellow.

The absorption spectra and general data for this indicator in the urea acetate–urea perchlorate system are shown in Figure 11 and Table VII. The evaluated equilibrium constant of 0.47 in this system indicates that it is somewhat less basic than urea in acetic acid.

Sudan III.



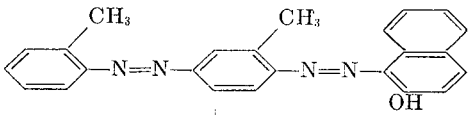
Sudan III is a member of the diazo dye series and has been used as an indicator in acetic acid by Spengler and Kaelin (*9*). It undergoes a color transition from yellow in basic solutions through red to blue in acidic solutions. The apparent color transition

from yellow to red is sharp, the transition from red to blue is somewhat gradual.

The apparent basicity of Sudan III in acetic acid is so low that in a 0.05*M* solution of urea perchlorate only about 10% of the indicator is converted to the acid form. For this reason it was necessary to use perchloric acid solutions per se in order to evaluate the indicator. Absorption spectra of the indicator in perchloric acid solutions are shown in Figure 12 and the general data are given in Table VIII. Evaluation of the equilibrium data indicates an equilibrium constant of approximately 740 for the reaction. Because the presence of very small amounts of water has a significant effect upon the apparent acidity of perchloric acid in the acetic acid system, and because only reasonable efforts were made to keep the system anhydrous, the constant of 740 is probably an approximate value.

In all the preceding systems the acids responsible in all cases for the color changes observed were not perchloric acid but the conjugate acid of the base present. With Sudan III and Sudan IV perchloric acid (acetic acid perchlorate?) acts as the acid. Even in these instances the presence of protophilic impurities such as water, which may appear in commercial grades of acetic acid, may introduce some doubt as to the exact nature of the effective acidic species.

Sudan IV.



Sudan IV (scarlet red) is also a diazo dye. The indicator undergoes a two-color transition from red (alkaline) to blue (acidic). As in the case of Sudan III it was found necessary to evaluate the indicator in solutions of perchloric acid per se.

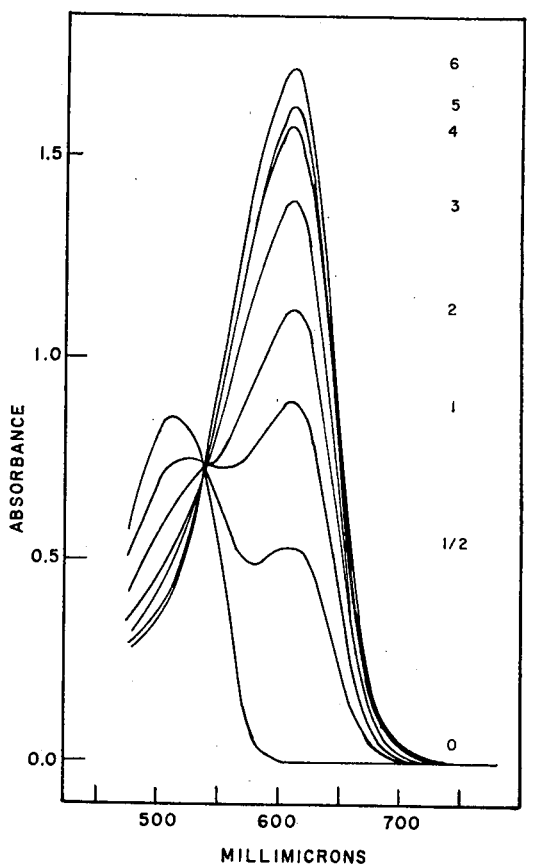


Figure 12. Absorption spectra of Sudan III

Solutions of perchloric acid in acetic acid

Absorption spectra of Sudan IV are shown in Figure 13; the general data are given in Table VIII. The equilibrium constant of the reaction between the indicator and perchloric acid was found to be approximately 525. Here again, the constant is a very approximate value because only reasonable efforts were made to maintain the system anhydrous.

POTENTIOMETRIC AND DILUTION STUDIES

Potentiometric determinations were carried out as described previously for all the indicator systems studied spectrophotometrically. Individual measurements are included in Tables I to VIII. As suggested recently (*6*) the response of a glass electrode to acetic acid solutions appears to be a measure of the acetate ion activity of these systems. The data for ephedrine and antipyrine, for example, follow the relationship already found by Higuchi, Danguilan, and Cooper (*6*) for sodium acetate–sodium perchlorate.

Table VIII. Interaction between Sudan III or Sudan IV and Perchloric Acid System in Acetic Acid

Solution	Molar Concn., $HClO_4$	E.M.F., Mv.	Absorbance	$\frac{IH^+}{I}$	K^a
Sudan III			613 mμ		
0	0.00000	..	0.00	...	...
1/2	0.00061	375	0.53	0.445	730
1	0.00152	382	0.89	1.07	700
2	0.00304	394	1.22	2.44	800
3	0.00608	404	1.39	4.22	690
4	0.0121	413	1.57	10.4	860
5	0.0244	423	1.62	16.2	670
6 (IH^+)	0.0547	..	1.72	...	..
Sudan IV			615 mμ		
1 (I)	0.00000	..	0.02	...	...
2	0.00304	394	0.93	1.49	490
3	0.00608	402	1.15	2.90	480
4	0.01216	414	1.32	5.91	490
5	0.0243	423	1.44	14.2	580
6 (IH^+)	0.0547	..	1.54	...	..

[a] For Sudan III, based on equilibrium Sudan III + $HClO_4 \overset{K}{\rightleftharpoons}$ Sudan III·$HClO_4$; for Sudan IV, based on equilibrium Sudan IV + $HClO_4 \overset{K}{\rightleftharpoons}$ Sudan IV·$HClO_4$.

Unlike aqueous buffer systems, the acidity of acetic acid solutions, determined potentiometrically, shows a definite concentration dependency. For all the systems investigated a tenfold dilution of an acetic acid buffer of a given relative composition resulted in an increase of approximately 30 mv. in the glass electrode potential. This is in agreement with the postulated relationship

$$\text{Acetate} = \frac{K(M^+Ac^-)}{K(M^+ClO_4^-)^{1/2}}$$

Dilution of acetic acid buffer solutions, on the other hand, produced no apparent change in the extent of their reaction with indicators. This behavior was spectrophotometrically established for all the indicators except the two Sudan colors. The theoretical basis of this is apparent from the nature of Equation 4. Because two reactant species yield two product species, the extent of reaction cannot be significantly affected by dilution.

GENERAL DISCUSSION

Comparison of Titrations in Acetic Acid and Aqueous Media. The data presented in the preceding sections show that indicator behavior during acid-base titration in acetic acid (or in any solvent of low dielectric constant) is substantially different from that commonly observed in water. Unlike aqueous titration, color changes in these systems appear to depend in part on the relative concentration and the degree of dissociation of the salt formed during titration—e.g., sodium perchlorate. In brief, the process can be considered to be the titration of a weakly disso-

ciated base with a weakly dissociated acid to yield a weakly dissociated salt.

Because of the nature of the titration reaction, certain basic principles observed in aqueous titrimetry are not necessarily applicable in solvents of low dielectric constant. In water the sharpness of the visual end point is largely dependent on the concentration of the strong acid or base used; therefore, the common practice is to employ reagent solutions of moderate strength and to use microburets for determinations of small samples. In acetic acid, however, the sharpness of the color change is often independent of the absolute concentrations (as indicated by the dilution studies). Because of this relatively dilute reagents may be used for small samples without significant loss in precision in many instances.

Basicities of Buffer. Although no serious efforts were made in these studies to determine precisely the relative basicities of the several buffer systems (thus the useful acidity range of the several indicators), approximate values can be gathered by perusal of Table IX, where estimated exchange constants of the systems are listed. The data listed are advisedly not the overall mean values but rather good guesses based on the more reliable measurements. From Equation 5 it is evident for the perchlorate-acetate systems that

$$\frac{K_{M_1}}{K_{M_2}} = \frac{\dfrac{K_{M_1+Ac^-}}{K_{M_1+A^-}}}{\dfrac{K_{M_2+Ac^-}}{K_{M_2+A^-}}} = \frac{\dfrac{K_{M_1+Ac^-}}{K_{IHA}}}{\dfrac{K_{M_2+Ac^-}}{K_{IHA}}} \qquad (7)$$

In Table X the relative basicity of the various buffer systems with the sodium system taken arbitrarily as unity is listed for the several indicators as calculated from Table IX. Where direct comparisons are possible, reasonable agreements are obtained with different indicators. From the table it is possible to approximate indicator interchange constants for a variety of systems if data for one are available.

However, the table is valid only for color changes involving conversion of the acetate (or acetic acid solute) form of indicators to their corresponding perchlorate form. It would appear that, if some other strong acid than perchloric acid was used throughout, the apparent relative basicity of the basic component would still be the same as given in the table. This follows from Equation 5. On the other hand, the relative basicity of the sodium sulfate–sodium acetate system, for example, cannot be determined directly by comparison in a similar fashion of the exchange constant of this system with that of sodium perchlorate–sodium acetate.

Although in these discussions acidities and basicities are expressed in terms of acetate concentrations, analogous relationships can be derived through use of other related parameters. Because at the time of writing of this paper, the autoprotolytic constant of acetic acid was not yet well established, it was felt that the use of the acetate was somewhat preferable.

Choice of Indicators. Quinaldine red and all the indicators listed below it in Table IX are suitable for titration of bases effectively as strong as ephedrine acetate or stronger. Because nearly all amines fall into this class, quinaldine red, for example, can be used rather widely in acetic acid. This is the indicator of choice especially where interfering weak bases such as urea may be present. For the determination of extremely weak bases Sudan III appears to be the indicator of choice. The magnitude of errors to be expected from each system can be anticipated from the data.

Table IX. Equilibrium Constants of Indicators in Acetic Acid Systems

	Buffer System[a]					
	E.HAc E.HClO$_4$	NaAc NaClO$_4$	NaAc Na$_2$SO$_4$	A.HAc A.HClO$_4$	U.HAc U.HClO$_4$	HClO$_4$
Ethyl red	10	45	3.7	450		
Pinacyanol	0.23	1.6		13		
4-Dimethylamino-4′-nitrostilbene				15		
2-Nitro-9(4′-dimethylaminobenzal)fluorene				7.3		
4-Dimethylamino-4′-sulfamylazobenzene				6.8		
Quinaldine red	2.7×10^{-2}	0.14		1.3		
4′-Dimethylaminobenzalrhodanine				0.39		
m-Nitro-*N*,*N*-dimethylaniline				0.42		
Brilliant Cresyl Blue				2×10^{-2}	1.8	
1-Naphtholbenzein					1.1	
Nile Blue A					0.47	
Sudan III						740
Sudan IV						520

[a] E = ephedrine; A = antipyrine; U = urea; Ac = acetate.

Table X. Basicity of Several Acetate-Perchlorate Salts in Acetic Acid as Determined with Several Indicators

(Relative to sodium system = 1)

Indicator	Ephedrine	Antipyrine	Urea
Ethyl Red	5	0.10	
Pinacyanol	7	0.12	
Quinaldine Red	5	0.11	
Brilliant Cresyl Blue		0.11[a]	0.001[a]
Mean	6	0.11	0.001

[a] Based on assumption that antipyrine system is 0.11 as basic as sodium system.

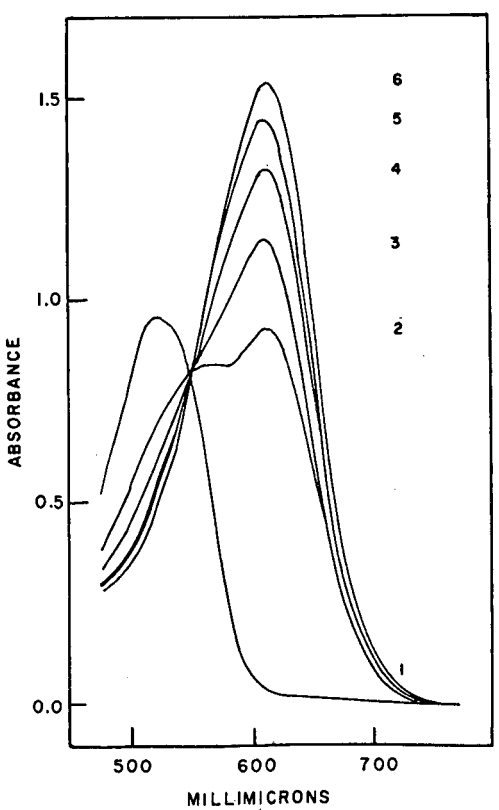


Figure 13. Absorption spectra of Sudan IV

Solutions of perchloric acid in acetic acid

ACKNOWLEDGMENT

The authors wish to acknowledge the support provided by the Research Committee of the Graduate School of the University of Wisconsin from funds supplied by the Wisconsin Alumni Re-

search Foundation. They are also grateful to Parke, Davis and Co. for financial help on the project.

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RECEIVED for review October 12, 1955. Accepted April 20, 1956. Based in part on thesis submitted by Joseph A. Feldman to the Graduate School, University of Wisconsin, August 19, 1955, in partial fulfillment of requirements for degree of doctor of philosophy.

Unique Polarographic Damping Circuit

For Selective Elimination of Current Fluctuations Due to Dropping Mercury Electrode

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The presence of current fluctuations due to the growth and fall of successive drops in dropping mercury electrode polarography causes some difficulty in measuring the height of polarographic waves for quantitative analytical purposes and is a severe limitation in all methods of derivative dropping mercury electrode polarography. A simple reliable filter circuit completely eliminates the drop oscillations from the recorded polarographic wave with a negligible effect on the wave form, although the half-wave potential suffers some displacement, the magnitude and direction of which are dependent upon the rate and direction of scanning. One has merely to extend straight-line segments when measuring the wave height of a filtered wave. The filtered wave height is directly proportional to concentration. The filter is being used for established polarographic concentration determinations and for derivative polarography.

THE dropping mercury cathode is widely used in polarography because it uniquely exploits the advantages accruing from the properties of a high hydrogen overvoltage on mercury and of a freshly renewed electrode surface. In addition to the useful polarographic information present in the electrical dropping mercury electrode current, there are interfering components due to the charging of the double-layer capacity, to the growth of diffusion current during the life of each drop, and to the severe transients caused by the fall of each growing drop. Though as applied to established concentration determinations, the polarographic technique is relatively rapid, much tedious time is spent measuring "mid-points" of individual drop oscillations before the wave form corresponding to the average current can be drawn and interpreted. An alternative method involves the measurement at the peak of each oscillation, which requires a rigid control of recorder response and damping conditions, as it is almost impossible to build any sort of polarograph that is capable of recording the oscillations with no damping whatsoever.

In differential or in derivative polarography involving two dropping mercury electrodes (*1*), it is necessary to synchronize the two drops and highly damp the resultant current. The fluctuation of the current of a single dropping mercury electrode is also a severe limitation in other methods of derivative polarography (*4*), because the instantaneous values of the derivative take enormous excursions. This necessitates heavy resistance-capacity (RC) damping, which in turn distorts the polarographic wave form and makes it impossible to record the true derivative of the polarographic wave. [In this paper the term RC damping is used to designate damping using the commonly used simple integrating filter consisting of a variable resistor in one leg of the recorder leads plus a capacitor directly across the input to the recorder (*2*). The time constant—i.e., the product of value of the resistor in megohms times the value of the capacitor in microfarads—determines the magnitude of the RC damping at any given frequency.] The result of compromise on this dilemma is to limit the field of application of derivative polarography, since with the required heavy RC damping, superior peak resolution is not realized between waves of close half-wave potential.

At this laboratory, a filter circuit has been developed which completely eliminates the drop oscillations from the recorded polarographic wave with a negligible effect on the form of the wave. Curves *a* and *b* of Figure 1 illustrate the inadequacy of ordinary RC damping. With a customary degree of RC damping (curve *a*), the frequencies corresponding to the average polarographic current are passed almost unattenuated but those corresponding to the dropping mercury electrode frequency and its rich harmonic content are only somewhat reduced. (The frequency selectivity of such a simple network is relatively poor.) Heavy damping (curve *b*) can eliminate the dropping mercury electrode components but not without reducing the magnitude of the lower frequencies, which must be passed unattenuated if

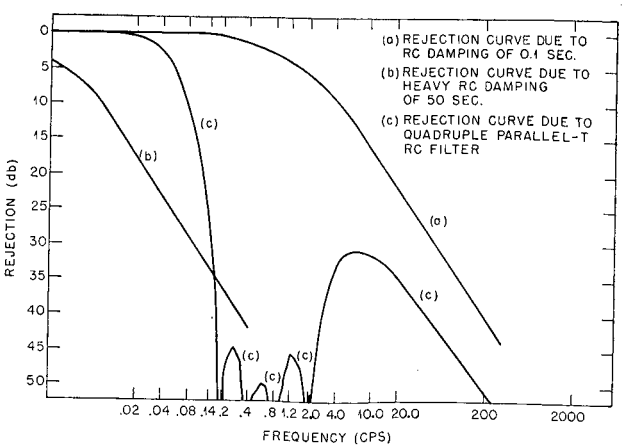

Figure 1. Comparison between RC damping and quadruple parallel-T RC filter damping

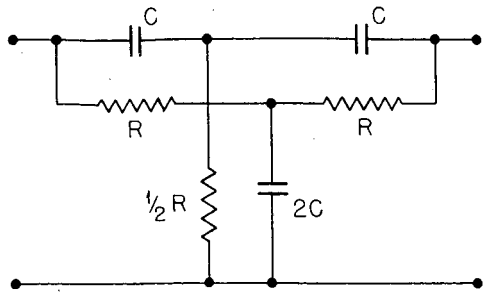

Figure 2. Circuit diagram of single-section parallel-T RC filter

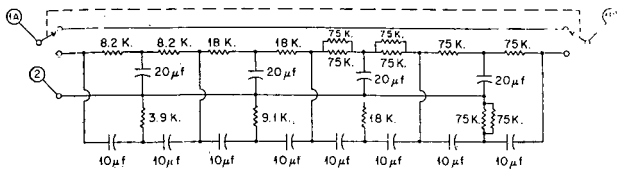

Figure 3. Quadruple parallel-T RC filter

the resulting wave form is to be undistorted. A frequency-selective filter is needed, for it is the value of the average currents during the life of consecutive drops that is of interest. For the frequencies corresponding to the fundamental and several harmonic values of the drop times ordinarily employed in polarography, inductance-capacity filters are impractical because the very large but high Q inductors needed cannot be obtained. The parallel-T RC filter (*5*, *6*) is frequency-selective and, fortunately, requires only inexpensive resistors and oil-filled capacitors.

The circuit of a single-section parallel-T RC filter is shown in Figure 2. This filter very strongly rejects a frequency, f_1 given by $f_1 = \frac{1}{2\pi RC}$, where the symbols are as indicated in Figure 2. The network should operate from a source whose impedance is small compared with R and into a load which is large compared with R.

DESCRIPTION OF PRACTICAL FILTER

A practical composite filter for polarographic use consists of four parallel-T RC filters which reject in order the tenth harmonic, fourth harmonic, second harmonic, and fundamental frequency of the dropping mercury electrode, followed by a simple RC section having a short time-constant. If the design null frequency of the fundamental parallel-T filter corresponds to the lowest drop time to be used, the filter will be equally effective for faster dropping rates as well, because the composite filter is essentially a low-pass network with an attenuation of at least 30 db. for all frequencies above the fundamental design frequency. The quadruple parallel-T RC filter, curve *c* of Figure 1, has a much sharper cutoff than does a simple RC damping filter. The combined filter is frequency-selective, rejecting the dropping mercury frequency and its harmonics, but passing virtually unattenuated the polarographic wave frequency. The use of a switch is suggested, so that the filter may be removed from the circuit whenever desired. It is sometimes convenient to record the oscillations—for example, when diagnosing troubles due to drop irregularities.

The circuit of a practical quadruple parallel-T RC filter is shown in Figure 3. It is inserted in series (1*A*, 1*B*) with the high impedance lead going to the simple RC filter network of the polarograph, and point 2 is returned to the ground bus. The impedance of the filter is high, but the introduction of it causes no difficulty in a polarograph using a high impedance recorder such as that described by Kelley and Miller (*2*). It has been installed in all polarographs used at this laboratory, and has received many hundreds of hours of usage with no malfunctions experienced. The high impedance of the filter restricts its immediate application to some commercial polarographs.

The current-measuring recorders of these polarographs can be readily modified to use the circuitry described by Kelley and Miller (*2*), but such a modification might also require other wiring changes, since the original design of many polarographs did not consider the problem of alternating current pickup, which can be disastrous to the operation of a high impedance recorder. It is impractical to design a low impedance parallel-T filter because oil-filled capacitors of the capacity required are prohibitive in size and cost. Electrolytic capacitors are unsuitable, because their capacity is somewhat variable, depending on their previous history, and their leakage resistance is undesirably low and also variable.

PERFORMANCE OF FILTER

The design null frequency of the quadruple parallel-T RC filter shown in Figure 3 is 0.2 cycle per second (a drop time of 5 seconds). The filter removes over 99% of the otherwise recorded excursions caused by the growth and fall of each drop of the dropping mercury electrode. The measured wave height is not identical with that obtained without the filter. However, without the filter, mid-points of recorded oscillations are often arbitrarily taken as indicative of average current (*3*). The output of this filter may indicate more nearly a true average of the current flowing at any time than do the mid-points of recorded oscillations without this filter. The recorded half-wave potential is retarded about 40 mv. (for a scanning rate of 0.1 volt per minute), but this known retardation is not a disadvantage for concentration determinations and may be taken into consideration for identification purposes. The precision with which a single filtered wave or with which replicate filtered waves may be measured is at least as good as that for the unfiltered waves. One has merely to extend recorded straight-line segments when measuring the wave height of a filtered wave. The relationship between wave height and concentration is linear, with no degradation observed due to the presence of the filter.

The relative effects of ordinary RC filters and of composite quadruple parallel-T RC filters terminated with a small amount

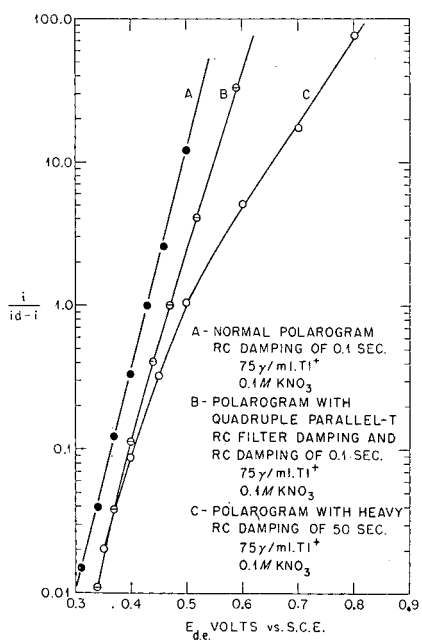

Figure 4. Effect of heavy RC damping and quadruple parallel-T RC filter damping on log $i/(i_d - i)$ *vs.* $E_{d.e.}$ relation

of RC damping are shown from different viewpoints in Figures 4 and 5. The data for both figures were obtained from polarograms of a solution containing 75γ per ml. of thallium(I) in 0.1*M* potassium nitrate. The observed relationship between log $i/(i_d - i)$ and $E_{d.e.}$ is plotted in Figure 4. Here it is seen that the distortion introduced by heavy RC filter damping is much worse than that due to the parallel-T RC filter. The polarograms from which the data of Figure 4 were taken are shown in Figure 5, which shows the relative effect upon the appearance of the recorded waves of heavy RC damping and of quadruple parallel-T RC damping.

Again, one may readily see that the effects of heavy RC damping are much worse than those of parallel-T RC damping and that very little distortion is introduced by use of this filter.

The retardation of observed half-wave potential due to the time lag of the parallel-T RC filter is seen clearly in the plot in Figure 4. For reversible reactions at the dropping mercury electrode one may make two polarograms with the filter in succession in first the forward and then the reverse direction of polarization. It is seen in Figure 6 that the arithmetic average of the two values of half-wave potential thus obtained is identical with that obtained in the normal direction of scanning with ordinary RC damping. This procedure has been used at this laboratory as an indication of whether a particular reaction is reversible.

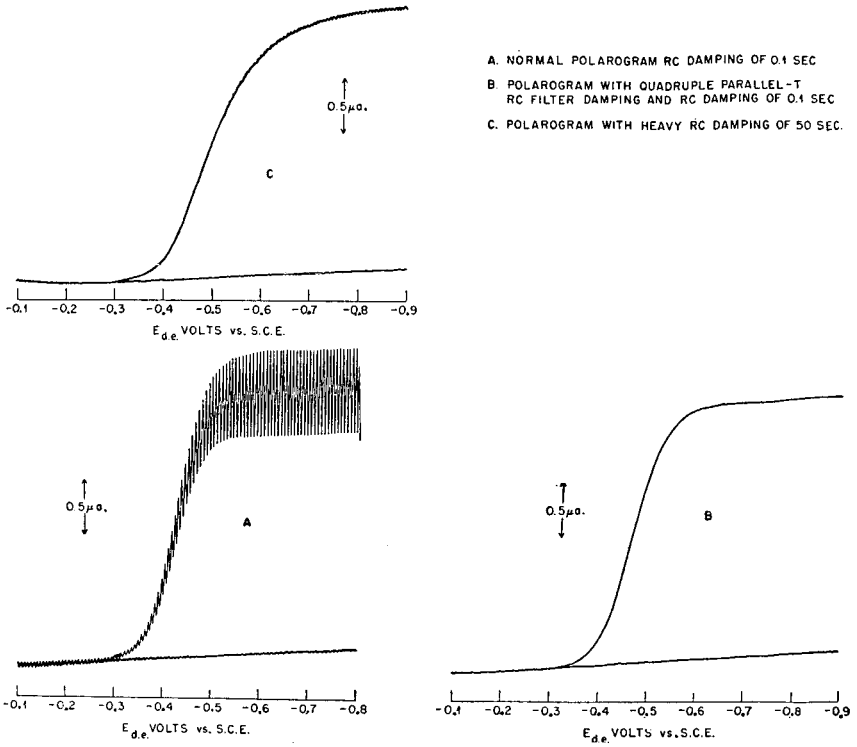

Figure 5. Effect of heavy RC damping and quadruple parallel-T RC damping on wave form

75 γ/ml. Tl^+
0.1*M* KNO_3

APPLICATIONS OF FILTER

Two principal advantages have been realized from the general use of the filter. There is a great reduction of time and labor in the analysis for concentration by established polarographic procedures, and the use of this filter drastically simplifies the attainment of a derivative polarographic wave. It has been successfully used as an aid for obtaining derivative waves by each of several techniques, the description of which is beyond the scope of this paper.

ACKNOWLEDGMENT

The authors wish to acknowledge the able assistance of Hugh H. Miller in obtaining the data presented in Figures 4, 5, and 6.

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RECEIVED for review December 12, 1955. Accepted March 24, 1956.

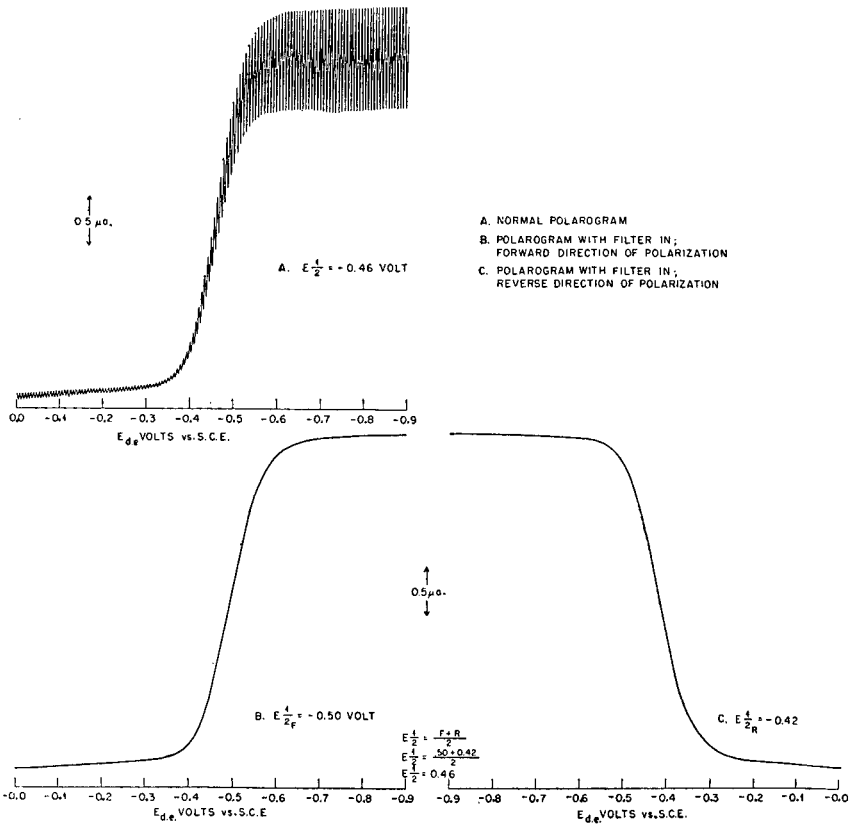

Figure 6. Effect of quadruple parallel-T RC filter upon half-wave potential (for reversible reactions)

100 γ/ml. Tl^+
0.1*M* KNO_3

Determination of Free Lime and Carbonate in Calcium Silicate Hydrates by Thermobalance

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The thermobalance has been used to determine free hydrated lime and carbonate with good accuracy in a number of calcium silicate hydrates. The results so obtained are more reliable than those obtained by the solvent extraction method for lime and the evolution method for carbonate. Amounts as low as 1.0% calcium hydroxide or carbon dioxide can be determined on samples of less than 0.5 gram.

IN THE preparation of all kinds of calcium silicate hydrate, a knowledge of the amount of free calcium hydroxide present is important because it influences the properties of the material. Free lime is soluble and may discolor or react with surrounding material. The formation of carbonate from free lime by atmospheric action may cause swelling with subsequent cracking. Carbonate is particularly important, because it is almost always present.

Free Lime. The standard ASTM method (*1*) appears to be satisfactory for determining free lime in portland cement but not in hydrated calcium silicates. Using this method, the writer found that the calcium hydroxide, which had been shown to be present in considerable amounts by the use of x-ray diffraction in the hydrated products from tricalcium silicate, β-dicalcium silicate, and tricalcium aluminate, was not extracted even after much longer periods than are normally used. The various extraction methods employed are normally suitable for dissolving calcium oxide, but when calcium hydroxide is present the results obtained are not too accurate. With hydrated cement compounds the difficulties are increased because the various methods do not sufficiently distinguish between the hydrated lime and the hydrated calcium silicate.

Various methods have been employed in attempts to determine free lime in hydrated calcium silicates. Bessey (*4*) has developed a calorimetric method. Schläpfer and Bukowski (*13*) extracted with ethylene glycol. Franke (*9*) used a mixture of acetoacetic acid and isobutyl alcohol. Kryagova (*11*) used thermal analysis to show the dehydration of calcium hydroxide in calcium hydrosilicate at 370° to 520° C. Brandenburg (*6*) used anhydrous barium chloride as an extraction accelerator. Assarsson and Bokström (*2*) studied the extraction methods and found that best results were obtained by conductometric titration with strong acid. Bernal, Jeffery, and Taylor (*3*), using x-ray and extraction methods for the determination of free calcium hydroxide, concluded with Bessey (*5*) that extraction methods are of doubtful significance.

One of the more accurate methods for which considerable claims have been made is that of Franke (*9*). In an attempt to prevent the solution of hydrated calcium silicate, considerable isobutyl alcohol, with or without anhydrous ethyl ether, is added to acetoacetic ester, the solvent for lime. The thermobalance method used in this present work was evaluated by comparing the results with those obtained using this extraction method.

Carbonate. It is difficult to obtain calcium silicate hydrates free from carbon dioxide. Taylor (*14*) says it is virtually impossible. His preparations contained amounts from 0.35 to 6.38%, with a hydrothermally prepared compound containing 2.66%. This is similar to a number of preparations made in this work. With considerable care, a carbon dioxide content of 0.5% was obtained. The carbonate determination can be made within limits by the acid evolution method. In most cases this requires a large sample as well as a blank determination. With low carbon dioxide content this blank may be of the same order as the carbon dioxide present. The limitations of the evolution method are such that poor and unreliable results are obtained on small samples and samples with low carbon dioxide content. Carbon dioxide obtained by heating the sample in a tube furnace tends to be high in some commercial materials because of small amounts of carbonaceous matter present. If the amount of carbon dioxide can be obtained by reference to a thermobalance curve, already made in order to determine the type of material and perhaps the amount of free lime present, the advantage is obvious. This possibility has been investigated in the present work.

EXPERIMENTAL

Preparation of Calcium Silicate Hydrate. These samples were prepared by heating equimolecular quantities of silica (pure hydrated silica or various types of diatomaceous earth) and hydrated lime (C.P. or commercial) in the presence of excess water at 80° to 85° C. for 2 to 20 hours, filtering, and drying to constant weight at 150° C. in a carbon dioxide–free atmosphere. The samples so obtained were stored in tightly capped bottles and placed in a desiccator. The carbon dioxide contents varied from 0.5 to 10% and higher. The samples with high carbon dioxide contents were purposely allowed to carbonate. In preparing these materials, the presence of free unreacted lime was normally avoided. This was checked by x-ray, differential thermal analysis, and the thermobalance.

Other samples were also prepared and used to compare the Franke extraction and thermobalance methods. These samples were obtained by hydrating pure tricalcium silicate under different conditions.

Use of Thermobalance. A simple thermobalance has been used by Clark and Sprague (*7*) to analyze lime products, but not in the presence of hydrated calcium silicate compounds. The thermobalance has also been mentioned by Greenberg (*10*) for the determination of free lime in calcium silicate hydrate I. The present work establishes this method on an experimental basis.

A Chevenard thermobalance (*8*) was employed. A heating rate of 8° C. per minute was used in an atmosphere maintained free of carbon dioxide and moisture. A further advantage of this method is that three determinations on materials in which organic matter is absent, or is present in negligible quantity, can be made at the same time—water, free lime, and carbonate, each independently of the other.

Effect of Sample Size, State, Composition, and Rate of Heating. The temperatures shown by the thermobalance are affected by the mass and state of the sample and the rate of heating. It has been stated (*12*) that it is difficult to fix the lowest temperature at which calcium carbonate decomposes on heating. This present work showed that it is not possible to predict accurately the decomposition temperature of hydrated lime. The differences in decomposition range are undoubtedly affected by the difference in temperature that exists between the sample and the thermocouple, which in this apparatus is near, but not in, the crucible holding the sample. This difference varies with the sample size, particularly at any inversion or decomposition temperature; it is larger with a larger sample. The thermocouple normally gives a temperature intermediate between that of the sample and that of the furnace.

Results obtained in this work emphasize the necessity of defining accurately the conditions under which runs on the thermobalance are made, particularly when comparisons are drawn.

Free Lime Determination. CALCIUM HYDROXIDE. To illustrate the effect of amount of sample on the temperature and rate

of decomposition, two thermobalance curves showing the decomposition of hydrated lime are given in Figure 1. (For reproduction purposes the curves in all the figures have been drawn with a much heavier line than is obtained with the thermobalance.) The decomposition range for the larger sample is 395° to 649° C., and for the smaller sample, 425° to 534° C.

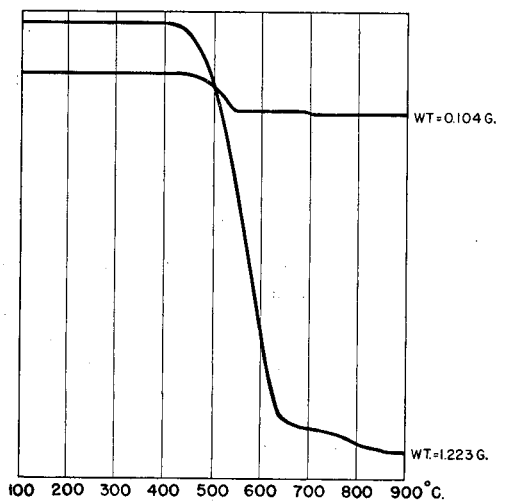

Figure 1. Thermobalance curves of calcium hydroxide

As a point of comparison, several handbooks give 580° C. as the decomposition temperature. In each case the amount of calcium hydroxide can be readily calculated from the amount of water lost between the two temperatures. The additional break in the curves between 650° and 830° C. is due to decomposition of the calcium carbonate present, resulting in the evolution of carbon dioxide. The amount of the latter can also be readily calculated.

CALCIUM SILICATE HYDRATE. Figure 2 shows curves for various calcium silicate hydrate preparations as well as the sample weights, which are included because the slopes of the curves depend upon them. These curves are entirely different from those of calcium hydroxide in that no definite dip occurs in the region where calcium hydroxide would decompose. There are no sharp breaks in the curves; all are similar, with the water of hydration coming off at different rates at different portions of each of the curves. The water of hydration comes off most slowly in the region where calcium hydroxide would give off water. In each case the slope of the curve is a straight line between about 375° and 650° C. The determination of free lime in such a product is based upon this fact. The dips above 600° C. are due to carbonate.

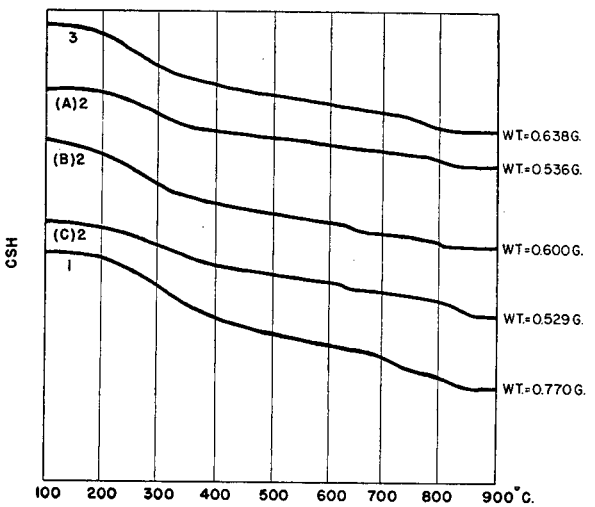

Figure 2. Thermobalance curves of series of calcium silicate hydrate preparations

Table I. Comparison of Thermobalance and Extraction Methods

Sample[a]	Calcium Hydroxide, % Extraction Method 1	Extraction 2	Extraction 3	Extraction 4	Thermobalance Method Total	Found	Calcd.
Blank	Nil				Nil		
$CaCO_3$, C.P.	0.01	0.00	..	..	0.01	Nil	Nil
$CaCO_3$, freshly precipitated	0.01	0.00	..	..	0.01	Nil	Nil
CSH I from C.P. materials	1.06	0.55	0.25	0.29	2.15	Nil	Nil
	0.94	0.69	0.34	0.29	2.26	Nil	Nil
CSH I from C.P. materials, highly carbonated	0.16	0.11	0.04	0.06	0.37	Nil	Nil
CSH I from diatomaceous earth	0.53	0.14	0.18	0.22	1.07	Nil	Nil
80% CSH I + 20% $Ca(OH)_2$, C.P.	20.28	0.60	0.41	0.21	21.50	19.11	19.38
	18.24	0.67	0.47	0.26	19.64	18.97	19.38
90% CSH I + 10% $Ca(OH)_2$, C.P.	10.63	0.48	0.39	0.30	11.80	9.56	9.69
	10.24	0.57	0.47	0.28	11.56	9.65	9.69
95% CSH I + 5% $Ca(OH)_2$, C.P.	5.55	0.51	0.43	0.32	6.81	4.82	4.84
	5.02	0.51	0.49	0.26	6.28	4.82	4.84
97.5% CSH I + 2.5% $Ca(OH)_2$, C.P.	3.32	0.51	0.48	0.22	4.53	2.39	2.42
	2.99	0.43	0.43	0.32	4.17	2.44	2.42
98.75% CSH I + 1.25% $Ca(OH)_2$, C.P.	2.16	0.50	0.44	0.27	3.37	1.16	1.21
	2.02	0.48	0.30	0.25	3.05	1.22	1.21
$0.5CaO.1.0SiO_2.xH_2O$, well reacted	0.30	0.17	0.10	0.13	0.70	Nil	Nil
	0.33	0.17	0.10	0.12	0.72	Nil	Nil
$2CaO.3SiO_2.H_2O$, well reacted	0.23	0.16	0.13	0.08	0.60	Nil	Nil
$3CaO.SiO_2$, C.P.	0.08	0.13	0.16	0.11	0.48	Nil	Nil

[a] CSH I = calcium silicate hydrate I.

CALCIUM SILICATE HYDRATE AND CALCIUM HYDROXIDE. Mixtures were carefully made of hydrated lime and calcium silicate hydrate in order to obtain quantitative determinations by means of thermobalance curves. Such curves are shown in Figure 3. The dip due to calcium hydroxide is readily seen in each case. By taking the vertical distance from the point at which the straight-line curve due to evolution of the combined water from the calcium silicate hydrate starts to change to the point where it resumes the calcium silicate hydrate decomposition drop, and calculating the calcium hydroxide from the loss in weight of water equivalent to this vertical distance, a good estimation of the amount of calcium hydroxide was obtained in all cases (Table I).

COMPARISON OF THERMOBALANCE AND EXTRACTION METHODS. Because only a small amount of material was available but a reasonable titration was desired, samples of less than 1.0 gram, and in most cases less than 0.5 gram, were used for the extraction method. The sample was extracted for 3 hours with a mixture of 3 parts of acetoacetic ester, 20 parts of isobutyl alcohol, and 5 parts of absolute ethyl ether, all by volume. The material was finely powdered and care taken to prevent absorption of moisture and carbon dioxide. After extraction the sample was filtered and washed with the same solvent mixture, then 20 ml. of absolute methanol and a few drops of bromophenol blue in-

dicator were added. The mixture was titrated with 0.1*N* hydrochloric acid to a definite yellow end point, which was sharp in every case.

In order to find out if all the titratable matter had been extracted, a further extraction was made. This was repeated a third and fourth time. By this means the efficiency of the method was examined on a large number of different samples. A blank run was also made using no sample.

Thermobalance curves were made of all the samples tested by the extraction method. The heating rate was 8° C. per minute.

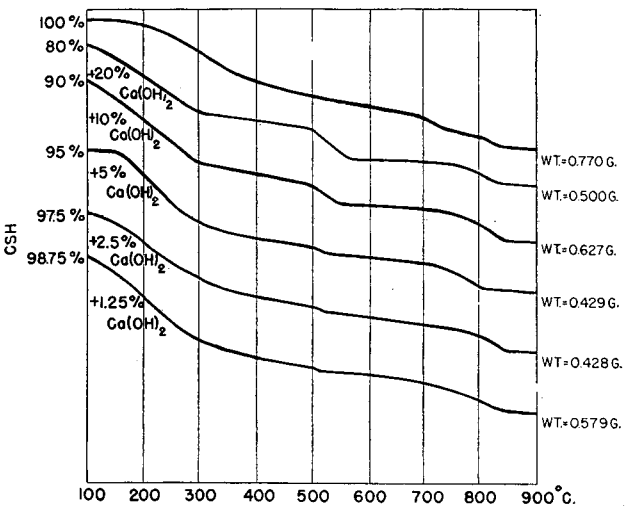

Figure 3. Thermobalance curves of calcium silicate hydrate with varying amounts of calcium hydroxide

The results are given in Table I, which shows that many calcium silicate hydrates in which no free lime was detectable by any known method gave significant amounts of extract. This was also the case when considerable excess silica was present and the reaction went to completion. The thermobalance method confirmed the fact that no free lime was present. With mixtures containing known amounts of free lime, extraction gave too high a figure percentagewise when around 20% was present. The deviation for the extraction method became greater when smaller amounts of free lime were present (the 1 to 2% range). The thermobalance method gave results which were 95 to 100% of the amount known to be present. Repeat results by the extraction method did not check each other as well as repeat results by the thermobalance method. This was particularly noticeable with the lesser quantities of free lime, those more likely to be met in practice. The amounts of the third and fourth extracts depend upon the type of material, and tend to become a fairly constant extraction factor for the specific material.

A large number of other hydrated materials from tricalcium silicate were analyzed by both methods. The results obtained confirmed the above observations.

It is concluded that, with calcium silicate hydrate materials that give off water very slowly in the region where calcium hydroxide decomposes, the thermobalance gives reliable determinations of the amount of free lime present. The Franke extraction method is definitely less reliable, particularly in the low ranges.

Carbonate Determination. Calcium Silicate Hydrate and Calcium Carbonate. The effect of carbonate with and without the presence of calcium hydroxide is illustrated by Figures 4 and 5. Figure 4 shows the effect of the presence of carbonate obtained during preparation. (The compound containing 10.28% carbon dioxide was prepared by deliberately allowing the wet material to carbonate in air.) The decomposition of samples with added calcium carbonate and calcium hydroxide is shown in Figure 5. Only parts of the curves are given.

Table II. Determination of Carbonate in Calcium Silicate Hydrate by Thermobalance

Sample	Carbon Dioxide, % Thermobalance	Carbon Dioxide, % Evolution	Ratio of Thermobalance to Evolution × 100
CSH	0.50	0.46	108.7
	0.78	0.77	101.3
	2.40	2.44	98.3
	2.65	2.69	98.5
	3.31	3.37	98.2
	3.86	3.83	100.7
CSH + $Ca(OH)_2$			
5% $Ca(OH)_2$	2.64	2.62	100.7
10% $Ca(OH)_2$	2.57	2.53	101.6
15% $Ca(OH)_2$	2.48	2.45	101.2
20% $Ca(OH)_2$	2.45	2.37	103.4
CSH, carbonated	8.90	8.95	99.5
	8.90	8.80	101.1
	10.28	10.23	100.4
CSH (low CO_2) + $CaCO_3$			
1% $CaCO_3$	1.04	0.95	109.5
5% $CaCO_3$	2.75	2.64	104.2
10% $CaCO_3$	4.98	4.82	103.3
20% $CaCO_3$	8.86	9.17	96.6
CSH, carbonated, + $CaCO_3$			
5% $CaCO_3$	11.52	11.80	97.6
10% $CaCO_3$	12.72	13.50	94.2
20% $CaCO_3$	16.03	16.88	95.0
CSH, carbonated, + $CaCO_3$ + $Ca(OH)_2$			
5% $CaCO_3$ + 5% $Ca(OH)_2$	11.37	11.35	100.2
10% $CaCO_3$ + 10% $Ca(OH)_2$	12.62	12.59	100.2
20% $CaCO_3$ + 20% $Ca(OH)_2$	15.42	15.04	102.5

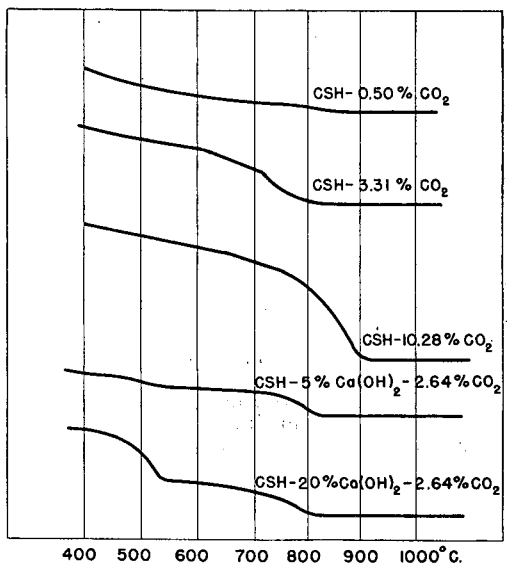

Figure 4. Thermobalance curves of carbonated calcium silicate hydrates

From the curves in Figures 4 and 5 the amount of carbonate present can be calculated in the same manner as for calcium hydroxide. When free lime is present, the straight portion of the curve for calcium silicate hydrate between 375° and 650° C. is broken but then continues in a straight line again, essentially parallel to the line just before the lime break. When carbonate

is present, this line dips because of evolution of carbon dioxide. If the vertical distance is measured between the points where this straight line commences to drop (evolution of carbon dioxide) and then becomes horizontal, the carbon dioxide content of the sample can be readily calculated. The figures obtained by this procedure check closely with values obtained on large samples by the evolution method. With small samples and those with small amounts of carbon dioxide, the thermobalance method is more accurate. Amounts as low as 0.5% on samples less than 0.5 gram, in the absence or presence of free lime, can be determined. When calcium carbonate is added, the amount obtained is equal to that present in the original sample plus that added. Table II illustrates this fact.

METHOD OF CALCULATION

Because decomposition curve for calcium silicate hydrate I betweeen 375° and 650° C. is essentially a straight line, any deviation from this straight line must be caused by the decomposition of the calcium hydroxide present. Calcium hydroxide starts to give off water at the beginning of this deviation and has been completely converted to calcium oxide at the end of it. At the point of deviation, the vapor pressure of the water present

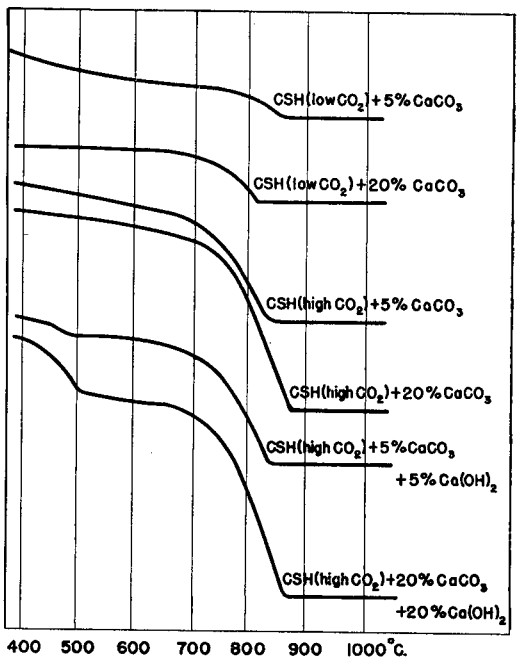

Figure 5. Thermobalance curves of calcium silicate hydrates showing effect of added calcium carbonate and calcium hydroxide

increases rather rapidly because of the much more rapid decomposition of calcium hydroxide. It would, therefore, be expected that the calcium silicate hydrate compound would lose water at a slower rate than normal. This normal rate is always slow at this temperature. A somewhat parallel effect is mentioned by Clark and Sprague (7). At this point the rate of decomposition approaches zero and remains constant until all the water from the free calcium hydroxide has been driven off. The fact that actual weight losses are close to theoretical throughout the range from 1 to 20% calcium hydroxide appears to verify the above assumption.

Thermobalance curves for calcium silicate hydrate I have a slight slope just before the carbon dioxide starts coming off. This indicates that the amount of water coming off at about 600° C. is small. As the thermobalance temperature rises continuously and comparatively rapidly, this state of affairs would be expected. It would be the more noticeable if, with carbonate present, some of the water is adsorbed by silica set free when the carbon dioxide originally reacted with the calcium silicate hydrate. Residual water from hydrated silica is notoriously difficult to drive off. It is possible that this small amount of water acts in a similar manner. Because the true carbon dioxide content can be readily calculated, it is evident that this residual amount of water is almost exactly counterbalanced by an equally small amount of carbon dioxide coming off with the water before the definite dip in the curve is observed. Because this is a dynamic decomposition method, such results are not unexpected. The presence of free lime and of added calcium carbonate does not interfere with the accuracy of the method.

Useful results have been obtained on samples other than the preparations upon which this work is based, using even less than 0.1 gram of sample.

SUMMARY

Free lime, ranging from 1.0 to 20%, can be calculated from thermobalance curves of calcium silicate hydrates, probably with greater accuracy than can be obtained by any previous method. Other hydrated calcium silicates, provided they give a gently sloping straight-line curve between 350° and 650° C. at a heating rate of 8° C. per minute, can also be analyzed for free calcium hydroxide by this method. In a similar manner carbon dioxide may be determined with greater accuracy than by any other gravimetric method, particularly on small samples and those with low carbonate content.

All results have been obtained on samples of less than 1 gram. Water, free lime, and carbonate in materials containing no appreciable amount of carbonaceous matter can all be determined from one thermobalance curve.

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RECEIVED for review February 15, 1956. Accepted April 3, 1956. Division of Analytical Chemistry, 129th Meeting, ACS, Dallas, Tex., April 1956.

Volumetric Determination of Primary and Secondary Nitroparaffins

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A rapid titrimetric method based on functional group analysis for the determination of individual primary or secondary mononitroparaffins depends upon the chlorination of the nitro compounds, using sodium hypochlorite in alkaline solution as the reagent. The excess reagent is determined by a standard iodometric method. The reagent consumed during chlorination is a measure of the nitro compound present when interfering compounds are absent. Because of differences in equivalent weights, the method cannot be used to analyze mixtures of several nitro compounds, a usual limitation of functional group analysis. Any compound that chlorinates in alkaline solution or is oxidized by the hypochlorite solution will interfere. The method has an accuracy within ±0.1% and a precision within ±0.05%.

THE mass spectrometer is the most useful means for analyzing mixtures of the several mononitroparaffins (nitroalkanes). However, when only a single nitroparaffin is present, a simple procedure based on functional group analysis can be used. This paper presents a rapid titrimetric procedure for the determination of individual primary or secondary nitroparaffins.

Published techniques for functional group analysis of these types of compounds include oxidation by chromic acid (*2*), modified Kjeldahl nitrogen determination (*4, 9*), reduction of the nitro group (*6–8, 12*), and, for nitromethane, potentiometric titration with sodium methylate using *n*-butylamine as the solvent (*3*). These procedures proved unsatisfactory for the routine determination of all primary and secondary nitroparaffins.

The chlorination of primary and secondary nitroparaffins can be explained as an electrophilic mechanism. All hydrogen atoms on the carbon attached to the nitro group can be replaced with chlorine when controlled amounts of an alkaline chlorinating reagent are used (*1, 5, 11*). The stoichiometric reaction is illustrated using nitroethane as an example.

$$CH_3CH_2NO_2 + 2NaClO \xrightarrow{NaOH} CH_3CCl_2NO_2 + 2NaOH$$

This reaction is the basis of the present analytical method. The excess chlorinating agent is determined by an iodometric method.

APPARATUS

Iodine flasks, 500-ml., glass-stoppered.
Volumetric flasks, 250-ml., glass-stoppered, short necked, Normax brand or equivalent.
Pipets, 5-, 10-, 15-, and 25-ml., Normax brand or equivalent.
Buret, 50-ml., Normax brand or equivalent.
Ice bath.

REAGENTS

Glacial acetic acid, ACS reagent grade or equivalent.
Potassium iodide solution, 20% aqueous solution.
Sodium hypochlorite, Clorox, 5.25% by weight sodium hypochlorite.
Sodium thiosulfate, 0.1*N*, standard solution.
Sodium hydroxide, ACS reagent grade pellets or equivalent.
Starch indicator, 0.5% aqueous suspension.

Hypochlorite Reagent A. Place 10 ± 0.1 grams of sodium hydroxide pellets in a 1-liter volumetric flask containing water. Cool, add approximately 145 ml. of Clorox solution, and dilute to volume with water. Store in an amber, glass-stoppered bottle. This solution is the 0.25*N* reagent (based on sodium hydroxide) for primary nitroparaffin analysis.

Hypochlorite Reagent B. Place 80 ± 0.1 grams of sodium hydroxide pellets in a 1-liter volumetric flask containing water. Cool, add approximately 145 ml. of Clorox solution, and dilute to volume with water. Store in an amber glass-stoppered bottle. This solution is the 2.0*N* reagent (based on sodium hydroxide) for secondary nitroparaffin analysis.

Nitroparaffin Standards. The preparation, purification, and characterization of the purity of the following nitroparaffins have been described (*10*).

Nitromethane (NM), 99.99 mole %.
Nitroethane (NE), 99.9+ mole %.
1-Nitropropane (1-NP), 99.9+ mole %.
1-Nitrobutane (1-NB), 99.96 mole %.
1-Nitro-2-methylpropane (1-N-2MP), 99.82 mole %. The impurity present was determined to be 1-nitrobutane.
2-Nitropropane (2-NP), 99.99 mole %.
2-Nitrobutane (2-NB), 99.9+ mole %.

PROCEDURE

Determination of a Primary Nitroparaffin. Weigh 0.6 to 0.7 gram of nitromethane or 1.2 to 1.4 grams of the higher primary nitroparaffins into a tared 250-ml. volumetric flask containing water. Dilute to volume with water and mix thoroughly until the nitroparaffins dissolve.

Place 10 ml. of the dilution in an iodine flask containing 25 ml. of hypochlorite reagent A. Prepare a blank from 10 ml. of water and 25 ml. of reagent A. Stopper flasks and allow to stand at room temperature for 15 minutes. Cool the flasks in the ice bath. Add 15 ml. of acetic acid and 15 ml. of potassium iodide solution, mixing after each addition. Allow to stand 10 minutes in the ice bath and titrate with 0.1*N* sodium thiosulfate solution to the starch end point.

Table I. Equivalent Weights of Nitroparaffins

Nitro Compound	Molecular Wt.	Chlorines Added	Equivalent Wt.
Nitromethane	61.042	3	10.174
Nitroethane	75.068	2	18.767
1-Nitropropane	89.094	2	22.274
1-Nitrobutane	103.120	2	25.780
1-Nitro-2-methylpropane	103.120	2	25.780
2-Nitropropane	89.094	1	44.547
2-Nitrobutane	103.120	1	51.560

Table II. Determination of Nitroparaffins

(Per cent by weight)

NM	NE	1-NP	2-NP	1-NB	2-NB	1-N-2-MP
99.97	99.98	100.04	100.02	99.97	99.98	99.96
99.98	99.97	99.99	100.02	100.01	100.00	99.94
99.98	99.98	99.99	99.98	99.98	99.97	99.95
99.97	99.89	100.02	99.98	99.99	99.98	99.96
99.96	99.98	99.98	99.97	99.97	100.02	99.96

Determination of a Secondary Nitroparaffin. Weigh 1.2 to 1.4 grams of a secondary nitroparaffin into a tared 250-ml. volumetric flask containing water. Dilute to volume with water and mix thoroughly until the nitroparaffin dissolves.

Analyze a 10-ml. aliquot of this solution as for primary nitroparaffins, but substitute hypochlorite reagent B for reagent A.

Calculations.

$$\frac{25(B - T)N \times \text{eq. wt.}}{W \times V} = \text{wt. \% NP}$$

Table III. Effect of Alkali on Analysis of Nitroparaffins

(Per cent by weight)

Nitroparaffins	Normalities of Reagents							
	0.00	0.05	0.10	0.25	0.50	1.00	2.00	3.00
Primary								
Nitromethane	99.99	100.02	100.01	99.97	99.90	96.82	90.57	84.91
Nitroethane	102.30	100.00	99.97	99.98	99.94	99.58	95.86	91.63
1-Nitropropane	99.18	100.03	99.99	99.98	100.03	99.18	97.06	94.02
1-Nitrobutane	99.23	100.04	100.04	100.04	99.99	99.88	99.11	98.79
1-Nitro-2-methylpropane	99.38	99.94	99.94	99.96	99.95	99.48	99.02	98.81
Secondary								
2-Nitropropane	13.81	109.95	103.99	102.55	101.28	100.19	99.99	99.99
2-Nitrobutane	9.26	105.78	104.11	103.50	102.01	100.78	99.98	100.01

where

B = ml. of thiosulfate required by blank
T = ml. of thiosulfate required by sample
N = normality of thiosulfate
W = grams of sample
V = ml. of aliquot

The equivalent weights of the seven nitroparaffins tested are tabulated in Table I.

APPLICATION

This method has been applied to the determination of the individual nitroparaffins as a criterion of purity and to process samples where a single nitroparaffin is present. It cannot be used in mixture of nitroparaffins because of the difference in equivalent weights.

Results of replicate analyses on high purity nitroparaffins are given in Table II.

EXPERIMENTAL

The influence of several variables on the quantitative applications of this reaction was investigated, including the concentration of alkali, the time and temperature of the reaction, and the stability of the chlorinated compounds in the alkaline reaction mixture.

Table IV. Effect of Time on Chlorination[a]

(Per cent by weight)

Primary		Time, Min.	Secondary	
NE	1-NP		2-NP	2-NB
99.78	99.14	5	99.87	99.82
99.98	99.98	10	99.99	100.00
99.98	99.99	15	100.02	99.98
100.04	100.02	30	99.99	99.98
99.98	99.99	45	100.04	100.00
99.98	100.02	60	100.02	99.98

[a] Primary compounds were analyzed in 0.25N reagent, secondary in 2.00N.

Concentration of Alkali. It was found that the concentration of alkali was critical, in the sense that the optimum concentration for the determination of primary nitroparaffins was not the optimum concentration for the secondary ones. The data on the effect of alkali, presented in Table III, indicate that the primary nitroparaffins react quantitatively in the range of 0.10 to 0.50N alkali. Secondary compounds react quantitatively in the range of 2.00 to 3.00N alkali. A 0.25N solution was chosen for the primary and a 2.00N solution for the secondary compound.

The high results for the secondary nitroparaffins, 2-nitropropane and 2-nitrobutane, in the lower alkali normalities (Table III) may be due to oxidation by the hypochlorite solution or to side reactions caused by structural differences. The chlorine derivatives of nitroethane, 2-nitropropane, and 2-nitrobutane are soluble in the reaction mixture. The other nitroparaffins precipitate after chlorination.

Time and Temperature of Reaction. The chlorination proceeds rapidly at room temperature. A study was made of the time needed for complete chlorination and of the effect of additional time upon the reaction, since the nitroparaffins themselves are sensitive to alkali (Table IV). While the chlorination appears to be complete in 10 minutes, a 15-minute reaction time was chosen to ensure complete chlorination.

Samples that were allowed to stand for 1 hour in the alkaline reaction mixture before acidification gave quantitative results (Table IV). The chlorinated nitroparaffins are stable in the alkaline reaction mixture.

DISCUSSION

A basic procedure is presented for the volumetric determination of any individual primary or secondary aliphatic mononitroparaffin. Tertiary nitro compounds do not react.

Because of differences in equivalent weights, mixtures of primary or secondary compounds cannot be analyzed by this method, a usual limitation of functional group analysis. All compounds that chlorinate in alkaline solution, or consume chlorine by oxidation of the hypochlorite solution, interfere.

To show the precision, accuracy, and stoichiometry of the chlorination reaction, the data presented have been necessarily limited to the analysis of high purity nitroparaffins.

The purity values for 1-nitro-2-methylpropane given in Table II are slightly higher than the 99.82 mole % determined by Toops (*10*). These high results are due to the presence of 1-nitrobutane as an impurity, which, having the same equivalent weight, is calculated as 1-nitro-2-methylpropane.

The data in Table II, when compared to the cryoscopic purities, show a standard deviation of 0.023%. The 95% confidence limit for a single determination is ±0.05%, providing precautions are taken to minimize loss of chlorine or iodine.

ACKNOWLEDGMENT

The authors wish to thank Emory E. Toops, Jr., for the preparation, purification, and characterization of the purity of all the nitroparaffins used in this study.

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RECEIVED for review February 29, 1956. Accepted April 18, 1956.

Isolation and Measurement of Uranium at the Microgram Level

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A double cupferron separation of uranium using extraction has been adapted to the micro level. Uranium(VI) does not extract in the first stage, which removes many potentially interfering elements. Uranium(IV), obtained in the residual aqueous solution by reduction at a mercury cathode, is simultaneously extracted as the cupferrate into ether, from which it can be re-extracted into nitric acid. A relatively simple one-piece glass apparatus is used for all operations. The uranium recovery at the milligram level in an initial 30-ml. sample was determined colorimetrically as 94%. With 0.03 to 0.13 γ of radioactive uranium-233 tracer and 20 γ of natural uranium as carrier, the recovery is 86%; the latter includes the additional step of electrodeposition of the uranium onto a platinum planchet prior to measurement by alpha counting, which is only 94% complete. The decontamination possible with this procedure was checked with 0.07 γ quantities of uranium-233 in the presence of high mixed fission product activities; 85% recovery was obtained, containing only 0.9% of the fission product alpha activity (assumed to be uranium).

WITH the increasing use of atomic energy for both military and peacetime uses, more attention is being given to means of determining and recovering the small amounts of uranium present in depleted reactor fuels. The disposal of radioactive wastes from atomic installations has created still another reason for developing means for determining and separating out minute amounts of radioactive heavy metals.

The present investigation is concerned with the separation and determination of milligram, microgram, and submicrogram quantities of uranium, including the recovery and assay of radioactive uranium present in admixture with large amounts of fission products. Because such decontamination may be of importance in removing uranium from fission products, an attempt was made to determine the manner in which the radioactivity is distributed in the procedure developed.

In view of the efficiency of liquid-liquid extraction, attention was focused on the separation of uranium by extraction—e.g., of the chelate species which it forms with organic molecules. Measurement at microgram and submicrogram uranium levels was made through the use of uranium-233 and alpha counting; at higher uranium levels, photometric measurement was utilized. Attention was focused on the development of a procedure, requiring simple equipment and only moderate amounts of time, applicable to very small amounts of samples, and adaptable to automatic or semiautomatic manipulation with the minimal introduction of chemical reagents and solvents.

BASIS FOR ANALYTICAL PROCEDURE

Uranyl ion forms a double salt, $UO_2NH_4(Cup)_3$, with cupferron (ammonium salt of *N*-nitrosophenylhydroxylamine) only from neutral solution; this salt is insoluble in organic solvents (*1, 4, 5*). A second, ether- and chloroform-extractable form appears to exist in acid media (*4*). As extraction procedures are particularly attractive for isolating microgram amounts of uranium from other elements, the statement (*10*) that "uranium and antimony are the only elements that will survive a double cupferron separation" becomes of particular interest.

In the double cupferron separation often used in analyzing uranium minerals (*6*), an aqueous solution containing the elements in their higher states of oxidation is treated with cupferron and the precipitated cupferrates are removed by filtration or extraction. After destruction of the organic matter in the aqueous phase, the latter is treated with a reducing agent to reduce uranium to uranium(IV) which is then precipitated with cupferron or extracted with an ether solution of cupferron. Some of the disadvantages of such a procedure for the present investigation include: the tedious destruction of organic matter; contamination resulting from the usual chemical procedures for reduction—e.g., use of zinc columns or liquid amalgams; and the fact that uranium ends up in an organic liquid.

Electrochemical reduction of the uranium seemed a logical recourse from the second objection. If this could be accomplished in the presence of cupferron, while simultaneously extracting the uranium(IV) cupferrate, as it was formed, into an organic layer, there would be no necessity for the intermediate step of destroying organic matter. It seemed reasonable to hope that the third objection could be overcome by extraction into aqueous nitric acid.

Antimony, plus some other elements, could almost certainly be removed by prior extraction of uranium oxinate from antimony(V) (*2, 9*). Uranyl ion may then be re-extracted into dilute sulfuric acid, which is a usable medium for the cupferron procedure.

Furman, Mason, and Pekola (*4*) showed that efficient extraction of uranium(IV) cupferrate into ether requires a 5% or more dilute sulfuric acid solution and at least a twofold excess of cupferron over the 4 to 1 theoretical requirement; they give distribution coefficients for eight cases with a figure of 88.4 applying for C_{ether}/C_{water} from 1.5*N* acid with a 10 to 1 ratio of cupferron to uranium present. The preparation and general properties of cupferron and its application as an analytical reagent have been summarized in a number of references (*7, 10,* pages 24–5; *14*). As a synthetic organic material, cupferron should not have an objectionably high natural uranium content; it is easily purified by recrystallization from methanol, and is stable at room temperature when stored in the absence of light and over ammonium carbonate.

The polarographic behavior of cupferron has been described (*8*). A polarographic survey (*13*) of the uranium-cupferron system indicated that the electrochemical reduction of uranium(VI) to uranium(IV) and/or (III) cupferrate would be possible in the presence of cupferron at a potential of about −0.3 volt relative to the saturated calomel electrode. The data (*4*) on the extraction of uranium cupferrates into ether indicated no difficulty in this regard. The re-extraction of uranium cupferrates from ether into aqueous nitric acid (with partial decomposition of the cupferron and oxidation of the uranium) seemed feasible.

In the procedure finally developed, the aqueous sample solution containing uranium(VI) is extracted with a solution of cupferron in an organic solvent, such as ether or chloroform, which removes certain metal chelate complexes. The remaining aqueous solution, which contains the uranium, is now electrolyzed at controlled cathode potential, while the same or a different organic solvent containing cupferron is added to form a separate upper layer. As uranium is reduced, it forms a stable chelate species with the cupferron and is extracted into the organic solvent. The metal ions, originally unextracted, are generally not now extracted; either the electrolysis does not reduce them, or their lower oxidation states do not form extractable species. The uranium(IV/III) cupferrate is then re-extracted

from the ether into 7*M* nitric acid. The nitric acid extract, after decomposition of organic matter with concentrated nitric and perchloric acids, is used for measurement of the uranium.

The uranium isolated, if present in milligram amounts, can be determined by photometric absorption. Extremely minute amounts of uranium are electroplated into a small platinum disk, whose radioactivity in terms of alpha emission is then measured with a flow counter.

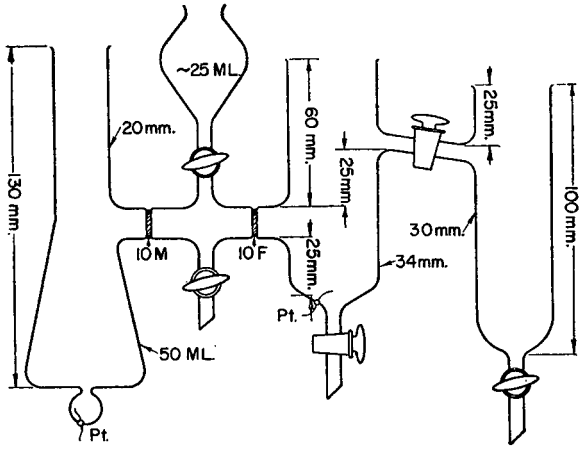

Figure 1. Apparatus for simultaneous reduction and extraction of uranium

All stopcocks Corning No. 7320

A simple apparatus was devised for the ready conduct of the entire sequence of operations: pre-extraction, simultaneous reduction and extraction, and re-extraction.

EXPERIMENTAL

Apparatus. The reaction cell construction is shown in Figure 1; the simple electrical circuit used is shown in Figure 2.

The electrolysis vessel, *C*, is protected from mercury ions diffusing from the working reference calomel electrode, *A*, by a medium glass frit between *B* and *C*, and a fine frit backed with an agar plug between *B* and *A*. Between runs, cell *C* is kept filled with saturated potassium chloride solution.

The first dozen runs of Table I were made using an apparatus similar to that of Figure 1, except that a tubular calomel cell, 25 × 95 mm., was used, the electrode area of which was one fourth that of the flask cell. With the tubular cell, the current flow in the presence of milligram quantities of uranium tends to build up a hard crust of calomel over the mercury, resulting in resistances as high as 700 to 1000 ohms.

The apparatus for the electrodeposition of uranium unto platinum disks or planchets and for alpha-counting measurement of the resulting uranium plates have been described (*11*). Beta activity was measured by a chlorine-quenched argon-filled Geiger-Müller counter (1.4 mg. per sq. cm. of window) with a Model 165 scaler; a scintillation well counter with a thallium-activated sodium iodide crystal and a Model 162 scaler was used for gamma-activity measurement of solutions (ca. 5 ml.) contained in a 13 × 150 mm. test tube. The scalers and counters are made by the Nuclear Instrument and Chemical Corp. For examination of the gamma-ray spectrum, a gamma-ray scintillation spectrometer (built in the Department of Chemistry, University of Michigan) was used through the courtesy of W. Wayne Meinke.

Uranium Solutions. Radioactive uranium-233 was obtained as a nitrate solution from Oak Ridge National Laboratory; isotopic analysis gave 1.0 to 1.5% U^{232} and 98.5 to 99.0% U^{233} (alpha 4.82 m.e.v.; $t_{1/2} = 1.68 \times 10^5$ years). The original solution (13 γ of U^{233}) was diluted to 100 ml., which was about 0.01*N* in nitric acid; 10 ml. of this solution was diluted to 100 ml. (0.01*N* in nitric acid); aliquots of the latter solution (1.3×10^{-8} gram of uranium per ml.) were used in the experimental work. The activity per microgram of uranium-233 was determined, as subsequently described, to be 7700 ± 88 counts per minute (background subtracted).

A uranyl sulfate solution (0.85 mg. of uranium per ml.), 0.18*M* in sulfuric acid, was prepared by dissolving 18.45 grams of the uranyl sulfate trihydrate in 12 liters of 1% sulfuric acid.

Fission Products. The gross fission products, obtained from Oak Ridge National Laboratory as U. S. Atomic Energy Commission Sample FP-P-1 (2.1 ml., 10 mc.), is a mixture of fission products present as nitrates in 5.4*M* nitric acid solution and prepared by separation from heavy metals which have been exposed for from 40 to 60 days in a reactor and cooled only a short time. The total solids were approximately 39.5 mg. per ml. (iron, ca. 2.6 mg. per ml.).

A sample (ca. 0.25 mc.), prepared by evaporating an aliquot of the fission products solution on a platinum planchet, was used for examining the gamma-ray spectrum. Three peaks were noted, due probably to cesium-137 (0.661 m.e.v.), cerium-141 (0.146 m.e.v.), and thulium-170 (0.076 m.e.v.). The standards run for calibration were cesium-137 (0.663 m.e.v.), chromium-51 (0.33 m.e.v.), and thulium-170 (0.085 m.e.v.).

The original fission products solution of 2.1 ml. was first diluted to 50 ml., 25 ml. of which was then diluted to 100 ml. (0.1*N* in sulfuric acid and 0.05*N* in nitric acid). From the latter, 1 ml. was diluted to 250 ml. (0.1*N* in sulfuric acid); finally, 5 ml. of the latter was diluted to 50 ml. (0.1*N* in sulfuric acid), which solution was used in the experimental work; 1 ml. of this solution gave 9207 ± 97 alpha counts per minute, 1707 ± 41 beta counts per minute, and 1890 ± 44 gamma counts per minute.

Reagents. All chemicals used were of C.P. or reagent grade unless otherwise specified. The ethereal cupferron solution used (200 to 300 mg. of cupferron per 50 ml.) was actually a hydrogen cupferrate solution; the ether and cupferron were mixed in a mixing cylinder with 5 to 10 ml. of 10 to 20% sulfuric acid and shaken until dissolution was complete.

PROCEDURES

Reductive Extraction. At the commencement of a run, bridge *B* is flushed through stopcock 2 by filling *B* with fresh potassium chloride solution from the funnel through 1. *C* is drained and rinsed; 1 is left open for a time to flush the frit. With 3 closed, 4 to 5 ml. of triple-distilled mercury is placed in *C*. About 30 ml. of uranyl sulfate solution (0.5 to 5 mg. of uranium and 0.5 to 1.5% in sulfuric acid) is added and a potential of −0.35 volt *vs.* S.C.E. is applied to the mercury. About 15 to 20 ml. of the ether cupferron solution is added. Stirring is adjusted at just over the minimal rate for efficient current flow (usually about 0.2 ma. flows without stirring and 1.2 to 2.6 ma. with stirring).

Table I. Recovery of Uranium at Milligram Level by Reductive Extraction with Cupferron

Group	No. of Runs	Uranium Taken, Mg.	Electrolysis Duration, Min.	Uranium Recovery, %
I	8	2 to 9	30 to 160	100 ± 20
II	12	0.1 to 4	50	94.0 ± 1.5
III	7	1 to 2	50	43 (av.)

Stopcock 1 is opened for about 30 seconds at approximately 5-minute intervals throughout the run to minimize any loss of uranium into the bridge. At 15- or 20-minute intervals, stirring is interrupted, the ether extract is bled through stopcock 4 into cell *D*, and 15 to 20 ml. of fresh ether-cupferron solution is added. Runs of 40- to 55-minute total duration appear to be adequate. Three increments of ether-cupferron solution were usually used, followed by a 5- to 10-ml. pure ether rinse at the conclusion of the run.

In some runs the current dropped to a low level soon after the requisite number of coulombs had passed for about a 3-electron reduction of the uranium present. In other cases, the current did not decrease, but discontinuance of the run beyond any point where twice the theoretical current had passed gave satisfactory uranium recovery. In the latter cases, a gray ether-insoluble, but alcohol-soluble precipitate (apparently a mercury cupferrate), was usually evident in the aqueous phase. The cur-

rent efficiency for the desired process appeared to be good in most runs.

The combined ether extracts may be re-extracted in cell *D* by inserting a clean stirrer, or they may be transferred with rinsing into a clean separatory funnel. Three extractions with 20 to 30 ml. each of 0.5*M*, 4*M*, and 0.5*M* nitric acid were adequate to re-extract uranium into aqueous solution.

Extraction and Measurement at Microgram Uranium Level. A solution of uranium-233 (10^{-7} to 10^{-8} gram) together with about 20 γ of natural uranium (as sulfate) was submitted to reductive extraction with cupferron for about 50 to 60 minutes. The uranium (IV/III) cupferrate was then re-extracted in cell *D* from the ether solution into three successive 15-ml. portions of 7*M* nitric acid. The combined nitric acid extract was evaporated to about 5 ml., treated with 25 to 30 ml. of concentrated nitric and 2 ml. of perchloric acid, and then evaporated to dryness.

Table II. Recovery of Uranium at Microgram Level by Reductive Extraction with Cupferron and Subsequent Electrodeposition

(30 γ of natural uranium carrier present)

Uranium-233 Taken, G.	Recovery Based on Counting, %
3×10^{-8}	74
7×10^{-8}	86, 84, 87, 86
	85, 87, 83
13×10^{-8}	88
Average recovery[a]	85.8 ± 1.3

[a] First run omitted.

The residue was digested with 10 ml. of 0.1*M* nitric acid for a few minutes; the solution obtained, after addition of about 10 γ more of natural uranium (as sulfate), was used for electrodeposition of the uranium unto a platinum planchet from an oxalate medium (*11*). A windowless flow counter with Q-gas was used for counting the alpha emission from the electrodeposited uranium (*11*).

The whole operation took about 4 to 5 hours. Each measurement of alphas from the samples was calibrated by counting a uranium oxide standard (National Bureau of Standards No. 836-5).

RESULTS AND DISCUSSION

Uranium Recovery at Milligram Level Using Photometric Measurement. The ferrocyanide colorimetric procedure in 0.05*N* nitric acid (*10*, pages 100–2) was used to evaluate uranium recovery at the milligram level (Table I). The organic matter in the final nitric acid extract must be destroyed by an initial evaporation with nitric acid and a second evaporation with nitric and perchloric acids; the solution is finally taken to dryness. No significant amount of sulfate ion may be present or results will be erroneously low.

In the first two runs of Group I no attempt was made to destroy organic matter. When it was recognized that enough undecomposed cupferron remained in the aqueous layer to jeopardize the colorimetric procedure, wet oxidations with nitric, perchloric, and sulfuric acids were employed. In runs 3 to 7 the aqueous solution was fumed with 2 ml. of concentrated sulfuric acid to a 2-ml. volume; the diluted solution (20 to 30 ml.) was neutralized with sodium hydroxide and then made about 0.05*N* in free nitric acid. On the suspicion that sulfate was bleaching the uranyl ferrocyanide color the solution in run 8 was fumed until only a moist residue remained containing not more than 0.2 ml. of acid. The apparent yield determined colorimetrically rose from 20 to 70%. As 2 ml. of sulfuric acid forms 5.5 grams of sodium sulfate, this amount of salt was added to a 2-mg. uranium sample which was checked colorimetrically. The absorbance corresponded to about one fifth of the appropriate value on the calibration curve. The uranium recoveries for the first eight runs on Table I are consequently estimated on the basis of this correction. In one run substitution of a spiral platinum cathode for the mercury pool gave an estimated recovery of only 75%.

The second set of 12 runs (Group II of Table I) was conducted similarly, except that during destruction of residual organic matter the samples were taken to dryness with perchloric acid and no sulfate was added. In three runs the complete double cupferron procedure was tested; the uranyl solutions were first twice extracted with 12-ml. portions of chloroform containing cupferron (250 mg. per 50 ml.), followed by a 10-ml. chloroform wash, and then electrolyzed, extracted with ether cupferron solution, and re-extracted into nitric acid solution; the average recovery was 85%. The over-all uranium recovery in four runs was also checked by the spectrophotometric 8-quinolinol procedure (*12*).

In a few experiments (Group III of Table I) an internal platinum anode was used with potentials from −1.2 to −2.5 volts. The poor uranium recovery indicates that reoxidation of the uranium occurs at an internal anode and that this modification is not feasible.

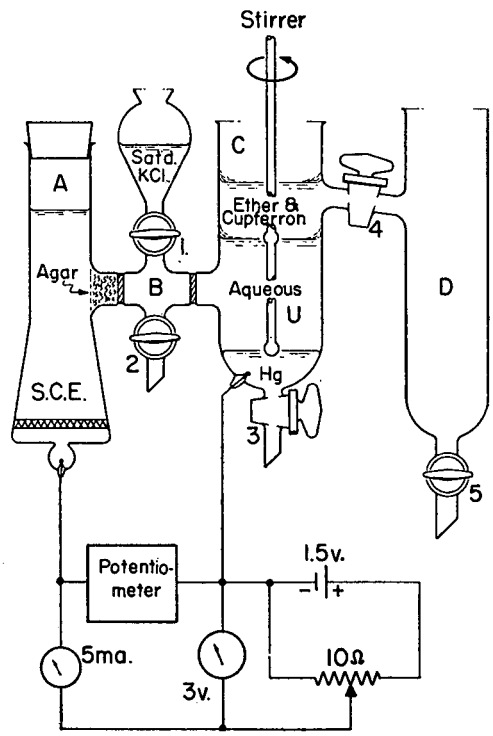

Figure 2. Electrical circuit for electrochemical reduction of uranium

Uranium Recovery at Microgram Level Using Tracer Technique. The average uranium recovery as measured by alpha counting was $85.8 \pm 1.3\%$ when the procedure was applied to samples containing 10^{-8} to 10^{-7} gram of uranium-233 and about 30×1.0^{-6} gram of natural uranium as carrier (Table II). In calculating the uranium-233 recovery (Tables II to IV and VI), correction was made for the alpha activity of the carrier based on 0.75 count per minute per γ of natural uranium (*3*) at 50% geometry. In one run, 7×10^{-8} gram of uranium-233 and 20 γ of natural uranium in 10 ml. of 1% sulfuric acid were first extracted twice with 12-ml. portions of chloroform containing cupferron (250 mg. per 50 ml.), followed by a 10-ml. chloroform wash.

In order to locate the 9% loss in these runs (a 6% loss is ascribable to the electrodeposition step prior to counting), several material balance runs were made. After the regular electrolysis and extraction procedure using 20 γ of carrier was completed, the catholyte, the cathode and bridge compartment walls, and the residual ether-cupferron phase from the final nitric acid extraction were checked for uranium activity. The data (Table III) indicate 4 to 6% loss in the residual catholyte and 2 to 3% loss in the residual ether-cupferron phase. Thus, about 93% of the total uranium activity could be traced. The final electrodeposition step alone for uranium-233 (in the presence of carrier), as reported previously (*11*), affords a 94% recovery of uranium.

Table III. Material Balance Runs for Uranium Recovery Using Uranium-233 Tracer

Uranium-233 Taken, G.	Uranium Recovery[a], %	Uranium Activity, %		
		Residual catholyte	Cathode and bridge compartment walls	Residual ether-cupferron phase
7×10^{-8}	85.3	4	0	3
7×10^{-8}	84.3	6	0	2
7×10^{-8}	86.4	5	0	2

[a] Loss of 6% in final electrodeposition step prior to counting.

Effect of Carrier on Uranium Recovery. The influence of the carrier on uranium recovery was determined by following essentially the procedure described, except that in one run about 10 γ of carrier was added before extraction and another 10 γ of carrier was added before electrodeposition, and in other runs, no carrier at all was added (Table IV). The calculations were made by comparing the activity of sample plates obtained after electrodeposition with that of a platinum disk prepared by evaporating 1 ml. of stock uranium-233 solution on it and then igniting to uranium oxide. The presence of 20 γ of carrier evidently enhances the uranium recovery.

Effect of Fission Products. In order to ascertain the behavior of fission products in the proposed procedure, carefully monitored runs were made, following the flowsheet outlined in Figure 3 and measuring alpha, beta, and gamma activities at appropriate locations. The results, in terms of the percentage of the total activity taken for that particular experiment, are given in Table V, A.

In two runs pre-extraction of the unreduced solution with cupferron was omitted and 10 γ of natural uranium was added as a carrier. The original 25-ml. solution (1% in sulfuric acid) was submitted to reductive extraction with cupferron, followed by addition of 10 γ of uranium carrier and electroplating. The activities, in terms of percentage of total activity taken, are shown in Table V,B.

From a comparison of the data in sections A and B of Table V, it may be deduced that:

About 20% of the fission product gamma activity and 1.3% of the beta activity are removed by the pre-extraction step.

Approximately 0.9% of the fission product alpha activity goes through the separation scheme (this quantity is logically attributable to alpha-emittive uranium present in the fission products).

Apparently the only major contamination of the uranium re-

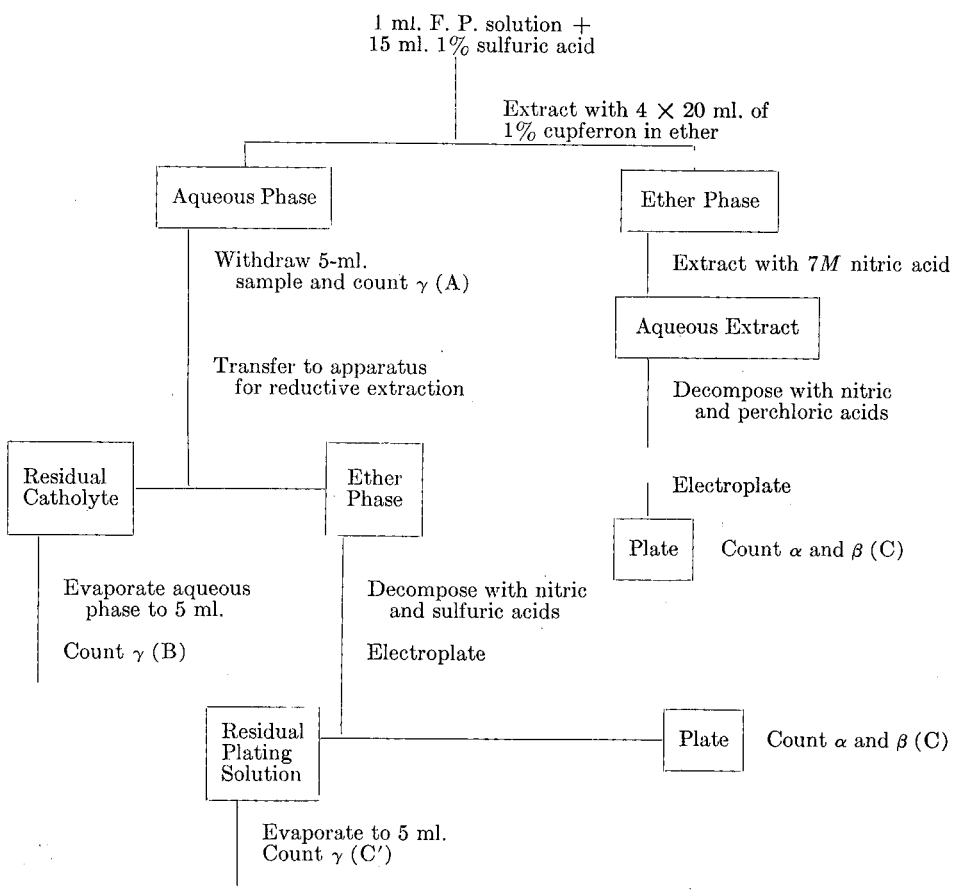

Figure 3. Procedure followed in determining distribution of fission products (F.P.) in proposed method for recovering minute amounts of uranium

Letters in parentheses are sample designations.

Table IV. Effect of Carrier on Uranium Recovery Using Uranium-233 Tracer

No.	Uranium-233 Taken G.	Uranium-233 Taken Activity, counts/min.	Natural Uranium Carrier, G.	Uranium Found Activity, counts/min.	Uranium Found Recovery, %
1	7×10^{-8}	810 ± 29	Nil	560 ± 24	69
2	7×10^{-8}	810 ± 29	Nil	520 ± 23	65
3	7×10^{-8}	810 ± 29	20×10^{-6}	710 ± 27	86

sulting from omission of the pre-extraction step (for this particular batch of fission products) is the 1.3% beta activity.

EVALUATION OF PROCEDURE

As only about 0.9% of the fission products (on the basis of counting alphas) can be plated after reductive extraction with cupferron in ether and as a recovery of 85.8 ± 1.3% was found for microgram quantities of uranium, it would appear that uranium can be recovered from admixtures with gross fission products and then determined by the proposed method. This was examined by mixing 7×10^{-8} gram of uranium-233 and about 10 γ of natural uranium carrier with increasing amounts of fission products to give an aqueous phase whose total volume (1% in sulfuric acid) was about 30 ml. The procedure of reduction, extraction, and plating was the same as before.

The results (Table VI) are corrected for carrier activity and a 0.9% recovery due to fission product alpha activity (Table V). Evidently, uranium-233 can be separated in about 85% yield from a mixture with gross fission products. Presumably, even more disproportionately larger ratios of fission activities to uranium might be adequately separable. This point is difficult to test rigorously, however, because of the sizable correction necessary for the alpha activity of the available fission product material. The average recovery in the presence of fission products (Table VI) checks well with the recovery found in their absence (Table II), but the scatter of the data is greater, because of the larger corrections applied (85.0 ± 8.0 and 85.8 ± 1.3%, respectively).

Table V. Effect of Fission Products on Proposed Procedures[a]

A. Recovery of Fission Products by Electroplating after Pre-extraction and Reductive Extraction with Cupferron in Ether (1 Ml. of Fission Products Solution Added[b])

No.	Activity in Aqueous Phase, % γ (A)	Activity in Aqueous Phase, % γ (B)	Activity in Ether Phase, % Plate (C) α	Activity in Ether Phase, % Plate (C) β	Activity in Ether Phase, % Plating solution (C′) γ, (residual)	Activity of Plate for Counting, % α	Activity of Plate for Counting, % β
1	79	72	0.84	0	9.5	1.12	0
2	86	70	0.80	0	7.5	0.98	0

B. Recovery of Fission Products by Electroplating after Reductive Extraction with Cupferron in Ether (No Pre-extraction)

No.	Fission Products Taken[b], Ml.	Natural Uranium Carrier Taken, γ	γ Activity, % In residual catholyte	γ Activity, % In residual plating solution	Activity of Plate, % α	Activity of Plate, % β
1	1	20	92	4	0.83	1.5
2	2	20	90	5	0.94	1.1

[a] Letters in parentheses refer to sampling locations noted in Figure 3.
[b] 1.00 ml. of fission products solution gives 9207 ± 97 α, 1707 ± 41 β, and 1890 ± 44 γ counts/min.

From the theoretical and manipulative viewpoints, the most important advance in the procedure developed is the introduction of an electrolytic process for changing the oxidation state of uranium in solution without introducing reagents or manual operations. In addition, a relatively simple one-piece glass apparatus has been devised which permits the entire sequence of operations prior to measurement to be performed with a minimum number of operations. The apparatus can be readily adapted to remote control.

The whole procedure takes about 4 hours. Although an average recovery of 94% characterizes the final plating step at the microgram level, the reductive-extraction and subsequent extraction steps are presumably equilibrium processes whose yield could be brought up to about 100% if the duration of reduction and the number of extractions were increased. With a total operating time of 5 to 6 hours, the over-all average recovery probably could be raised from 85 to 94%.

The recoveries obtained are satisfactory, as they are reproducible and, after correction for loss, would permit an accuracy good to at least 10% at the microgram level and about 2% at the milligram level.

Table VI. Separation of Submicrogram Amounts of Uranium-233 from Gross Fission Products

(20 γ of natural uranium added as carrier)

No.	Fission Product Solution Taken[a], Ml.	Uranium-233 Taken γ	Uranium-233 Taken Alpha activity, counts/min.	Residual Catholyte γ Activity, %	Activity of Plate α counts/min.	Activity of Plate β⁻, % recovery	Uranium-233 Recovered, %
1	1	0.07	500 ± 10	90	500 ± 22	0.9	80.4
2	1	0.07	500 ± 10	93	510 ± 23	1.2	82.5
3	5	0.07	500 ± 10	96	825 ± 29	1.0	79.0
4	5	0.07	500 ± 10	94	800 ± 28	1.5	74.0
5	10	0.08+	600 ± 11	94	1440 ± 38	2.3	99.8
6	10	0.08+	600 ± 11	95	1410 ± 38	1.8	94.2

[a] 1.00 ml. of fission products solution gives 9207 ± 97 α, 1707 ± 41 β, and 1890 ± 44 γ counts per minute.

The several stages of separation used in the present procedure—i.e., removal of elements in the pre-extraction stage, reduction of elements into the mercury cathode, removal of uranium in the reductive extraction step, transfer of uranium from ether to aqueous nitric acid, and the electrodeposition step, plus the selectivity of the photometric or radioactive counting measuring step—should provide the desired specificity for the determination of uranium in the presence of other elements.

ACKNOWLEDGMENT

The authors wish to thank the Air Force Cambridge Research Center which helped support the work described, and John L. Griffin and Herman Wissenberg for help with some of the experimental work.

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RECEIVED for review February 16, 1956. Accepted April 16, 1956.

Automatic Oxidimetric Micromethod for Uranium

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A method has been developed which uses a chemical and mechanical system for the rapid and convenient determination of 10 to 100γ quantities of uranium. Microliter amounts of standard ceric sulfate are automatically delivered to reduced sample solutions and the volumes are recorded on the chart of a recording potentiometer. Replicate titrations of 0.5-μmole quantities of uranium show a standard (95%) deviation of ±1.2%. The relative error for smaller samples is proportionally larger, and, therefore, the method is not recommended for samples containing less than 10 γ of uranium. The procedure should be helpful in dealing with pure solutions of elements which are conveniently determined by oxidimetric titration, such as iron and vanadium. The mechanical features of the apparatus may be altered to conform to various requirements both as to expected sample sizes and redox systems.

THE determination of from 10 to 100γ quantities of uranium is usually accomplished by colorimetric methods, the accuracies of which are at best limited to those of the readings obtainable on a spectrophotometer. In addition, such methods are generally inconveniently dependent on pH, reagent stability, and the like, and the required sample preparations are often tedious and time consuming. For samples containing larger amounts of uranium the oxidimetric titration of reduced strongly acid solutions is rapid, convenient, and accurate, and the usual standard oxidimetric reagents are stable indefinitely. Application of the latter method to less than milligram quantities of uranium would therefore be advantageous, provided the equipment necessary for the retention of the desired accuracy were not unduly cumbersome, costly, and operationally complex. A chemical and mechanical system which meets these requirements is described here.

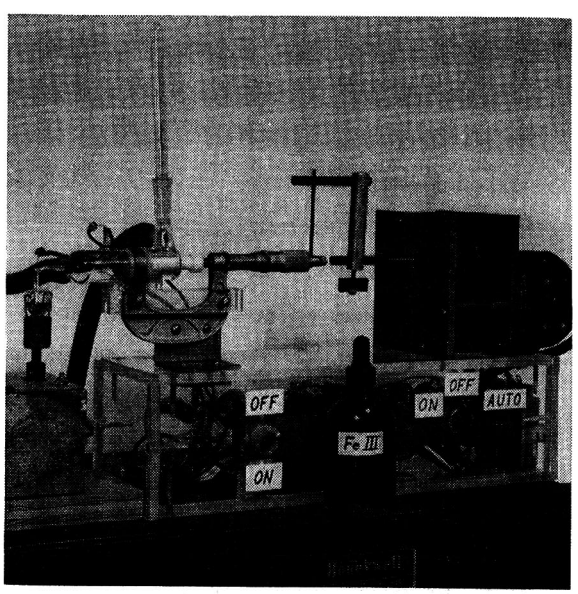

Figure 1. Automatic titration equipment

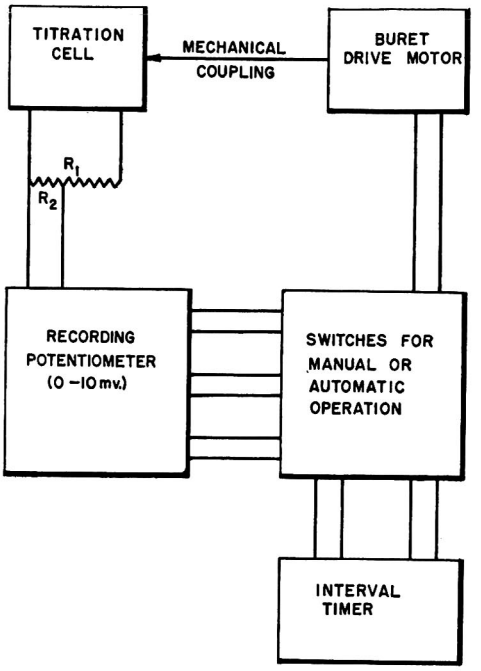

Figure 2. Block diagram of electrical system

$R_1 \approx 1$ megohm
$R_2 = 3900$ or 8200 ohms

There have been several other investigations dealing with the titration of small amounts of uranium. The methods described by Rodden (*7*) depend on the use of miniature Jones reductors and colorimetric end point indicators, and would appear to have a lower limit of about 0.1 mg. of uranium with an associated accuracy within ±2%. Disadvantages inherent in these methods include the large volume increases necessitated in washing the amalgam, the high blanks resulting from peroxide formation (*9*), and the use of indicators. Indicator blanks can be entirely eliminated by potentiometric end point detection, and an automatic microtitrator described by Kelley (*4*) is reported to titrate 50γ samples of iron (the approximate chemical equivalent of 0.1 mg. of uranium) with a standard (95%) error of ±1.2%.

The use of chromous and titanous ions for the initial uranium reduction has been reported (*7*), and offers the best means of retaining small sample volumes. With this modification and the use of ferric sulfate for the intermediate oxidation instead of ferric chloride, the present procedure is essentially that of Kolthoff and Lingane (*5*). Hahn and Kelley have described a similar method in which interference from small amounts of iron (up to a maximum of about half the weight of uranium present) is removed by complexing with 1,10-phenanthroline (*3*). Ceric sulfate was chosen as the standard oxidant because of its well-known stability in 0.5 to 1*M* sulfuric acid (*10*) and because this oxidant establishes a stable potential against inert electrodes such as platinum.

EXPERIMENTAL

Materials. Standard uranyl sulfate solutions in 1*M* sulfuric acid were prepared by the usual methods from black oxide (U_3O_8) equivalent to National Bureau of Standards material.

Ceric sulfate (G. Frederick Smith Chemical Co.) was prepared as a 0.1*M* solution in 0.5*M* sulfuric acid in order to keep the density of the titrating liquid below that of the samples. This limited mixing in the buret tip, which dipped into the samples (1*M* in sulfuric acid) during the runs, to true diffusion rather than the extremely rapid thermal and gravitational convection processes which might otherwise have occurred. Chromic sulfate, 0.1*M* in 0.1*M* sulfuric acid, was stored in a Jones reductor with a capillary tip and an amalgam bed measuring approximately 30 × 1 cm. The effluent chromous solution was used dropwise directly from the reductor. Ferric sulfate, 0.1*M*, was made in 1*M* sulfuric acid and stored in a small reagent bottle equipped with a dropper cap for convenience.

Apparatus. An automatic titrator was developed for this determination which embodies, with several simplifications, the features described by Robinson (*6*). The apparatus includes a motor-driven microburet (*1*), synchronized with a 0- to 10-mv. recording potentiometer, and an interval timer. The physical arrangement is shown in Figure 1, and a block diagram of the electrical system is shown in Figure 2. The buret drive was geared such that with the motor on continuously, titrant was ejected at the rate of about 1 μl. per minute, during which time the chart paper, graduated in 0.1-inch divisions, moved a little less than 1 inch. The titration cup, with a total capacity of about 1 ml., was cut from the end of a 15-mm. test tube. During a run, the cup was rotated at 300 r.p.m. with the buret tip (inside diameter, 0.05 to 0.1 mm.) and a platinum electrode dipping well into the sample solution at one side of the cup. A second platinum wire sealed into the buret completed the cell. The internal resistances of the latter varied from 20 to 50 kilo-ohms; its output was therefore shorted across a 1-megohm voltage divider from which the recorder read potentials corresponding to 3.9 or 8.2 kilo-ohms, depending on the desired scale coverage.

A mercury switch on the recorder was set to turn on the interval timer at the reproducible potential corresponding to the point of unit slope on the titration curves between the horizontal plateaus and the nearly vertical end point breaks. The timer then turned on the buret motor and the recorder chart drive for 1 second and off for 5 seconds, repeatedly, until the end point was completed. This cycle provided sufficient equilibration without undue loss of speed.

Procedure. The following procedure was used to obtain the data reported below, except where otherwise noted.

Set up the apparatus as shown in Figure 1 and allow a 2-minute warm-up for the recorder. Pipet 0.5 ml. of a solution from 10^{-4} to 10^{-3} *M* in uranyl sulfate in 1*M* sulfuric acid (about 12 to 120 γ of uranium) into the titration cup. Turn on the stirring motor and add 1 drop of chromous sulfate solution from the Jones reductor. Turn the operating switch to the manual "on" position until the potentiometer needle has travelled 80 to 90% of the full-scale distance to the right, then turn the switch back to "off." Allow 10 minutes for air oxidation of uranium(III) and chromium(II), then add 1 drop of ferric sulfate solution and turn the operating switch to "automatic." After the recorder has plotted the ferrous-ferric end point, turn off the operating switch and the stirring motor. The vertical distance on the chart paper between the point at which automatic operation was started and the mid-point of the iron break is proportional to the total amount of uranium in the sample.

The precision of delivery of a standard 1-ml. measuring pipet (Mohr type) can be increased considerably by drawing out the tip such that the last 1 to 2 cm. is of 0.1- to 0.2-mm. inside diameter and 1- to 2-mm. outside diameter. This slows the rate of delivery effectively and, more important, limits liquid pickup after delivery to a reproducible minimum.

With the 8200-ohm resistance in the voltage divider, the +1.6-volt potential of the titration cell immediately after addition of chromous ion is off the scale of the recorder. Titration curves showing the complete process were obtained with the 3900-ohm resistance. For routine determinations it is unnecessary to observe the initial reduction and aeration steps fully, and use of the 8200-ohm resistance results in a highly magnified iron break. This initial temporary operation of the system serves the purposes of cocking the mercury switch which turns on the interval timer as the ferrous-ferric end point is approached, and of providing an immediate visual check on proper operation of the apparatus and complete reduction of the sample.

A titration requiring 10 μl. of oxidant (equivalent to about 120 γ of uranium) is completed in about 20 minutes, including the aeration step. This time consideration is the reason for indicating the upper limit of uranium. The system is capable of titrating much larger quantities, but because the time required for a single determination is roughly proportional to the amount of uranium present, it is desirable to take only enough uranium to provide the necessary accuracy.

DISCUSSION

It was originally intended that the results of this determination would be obtained from the difference between the two end points corresponding to the oxidation of uranium(III) and chromium(II) (first) and of iron(II) (second). A plot of such a titration is shown in Figure 3, *A*. In this run the buret drive was operated continuously in the hope that overshooting at the breaks would be cancelled, or nearly enough to provide the desired accuracy.

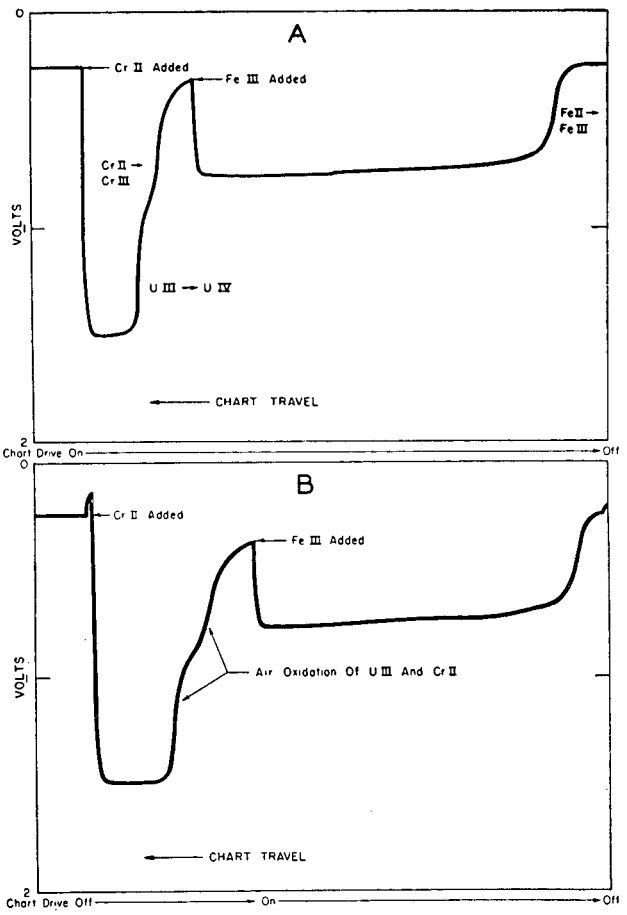

Figure 3. Comparison of first end points

0.5 μmole of uranium in 0.5 ml. of 1*M* sulfuric acid
A. Buret drive on continuously
B. Buret drive turned on at time of adding iron(III)

The results of these runs were generally poor, however, with regard to both precision and linearity with respect to total uranium. For the run shown in Figure 3, *B*, the buret drive was not turned on until air oxidation of the uranium(III) and chromium(II) was complete; there is no great difference in the shape of these first breaks from that shown in Figure 3, *A*. Thus, it became apparent that no cancellation of overshooting effects could be expected. Therefore, the interval timer was installed and the procedure given in the preceding section adopted. Figure 4 shows a series of runs at varying uranium levels obtained by this procedure.

The results of a large number of replicate determinations, conducted primarily to test the buret, are given in Table I. The buret had an inside diameter of $^3/_8$ inch (precision bore glass tubing) and a total possible piston travel of 1 inch. More than 100 determinations involving 10 μl. quantities of titrant each can therefore be made at one filling and it was necessary to check the linearity of the bore over a considerable distance. A statistical analysis of the results shown in Table I using the F test (*11*) indicated that the data could be pooled, and the standard 95% confidence interval deviation for a single determination at this uranium level was found from the pooled set to be ±1.2%.

Table I. Chart Inches for 0.500-μmole Samples of Uranium

Buret Initially Full	Buret Half Full	Buret Nearly Empty
8.46	8.53	8.44
8.53	8.49	8.46
8.42	8.52	8.53
8.54	8.56	8.51
8.48	8.44	8.58
8.42	8.63	8.52
8.43	8.48	8.54
8.47	8.55	Av. 8.51
8.52	8.41	
8.48	8.60	
8.48	8.50	
8.54	Av. 8.52	
8.48		
8.49		
8.56		
Av. 8.49	Grand av. 8.50	

The results of several runs at other uranium levels are shown in Table II. Linearity with respect to total uranium is exhibited, and it is qualitatively apparent that the absolute deviations are comparable to those shown in Table I. As was pointed out in the preceding section, therefore, the per cent accuracy of a given determination is proportional to the amount of uranium present. On this basis the titrations of 12γ quantities of uranium compare favorably in accuracy with both the colorimetric and fluorometric methods in this range.

Table II. Chart Inches for Varying Amounts of Uranium

Uranium Taken, μmole		
0.250	0.100	0.050
4.23	1.72	0.86
4.25	1.67	0.84
4.26	1.69	0.83
4.24	1.72	0.87
4.25	Av. 1.70	0.83
4.22		0.84
4.25		Av. 0.85
4.26		
4.32		
4.25		
4.24		
Av. 4.25		

This method is not recommended for samples containing less than 10 γ of uranium. Such samples give readable titration curves, but the per cent error becomes extremely high (about 25% for 5 γ) and the fluorometric method is at least as good as and usually better than this at these levels.

The sources of error inherent in this method can be divided into two categories, chemical and mechanical. The former includes (*a*) variations in potentials caused by differences in the sample solutions, (*b*) air oxidation of uranium(IV) after the addition of chromium(II) and before the addition of iron(III), and (*c*) interfering substances. Of these, (*a*) has not been observed and (*b*) was shown to be unimportant by aerating 0.05- and 0.5-μmole samples for 10, 20, and 40 minutes before adding iron(III); the results were in accord with those shown in the tables. The effects of interfering substances on this titration have been thoroughly investigated (*2*, *5*, *7*, *9*). The present method was developed specifically for pure uranium samples; it would probably be applicable to any unknown in which metals reducible by chromous ion are either absent or removable. This is particularly true in the case of iron, although it should be borne in mind that the tolerance levels for such interfering ions may be unusual at these uranium levels, especially with regard to the possibility of catalysis of the air oxidation of uranium(IV).

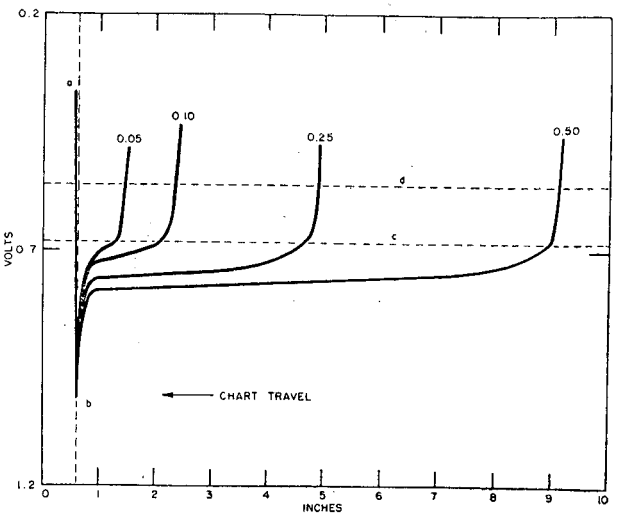

Figure 4. Titration for varying amounts of uranium

Amount of uranium given at breaks as μmole in 0.5 ml. of 1M sulfuric acid
a. Add chromium(II)
b. Begin titration after aeration
c. Potential at which incremental operation is automatically triggered
d. Potential at which distance from base line defined by *b* is read

The only commonly encountered anion which could give difficulty is phosphate (chloride and nitrate should be removed by fuming with sulfuric acid). If phosphate is present, the sulfuric acid concentration should be raised to 4 to 5M to avoid precipitation of uranium(IV) phosphate (*8*).

Mechanical errors include pipetting errors, temperature changes affecting the volume of the titrating liquid or the metal parts of the buret, variations in the length of the Teflon piston caused by squeezing and relaxation, differences in coast time between the buret drive and the chart drive during the incremental addition cycle, chart paper length changes due to humidity variations and/or binding in the recorder, and variations in the buret bore. Of these, pipetting errors can be controlled by using the same pipet for standards and unknowns; temperature changes affecting the volume of the titrating liquid were rendered negligible by a water jacket, kept at 25.00° ± 0.01° C., for the buret.

The remaining sources of error are not easily subject to evaluation. If any of them were sufficient to lead to consistent errors greater than those apparent from the deviations noted in Tables I and II, then an equally consistent compensating effect must have been operative. This is regarded as highly unlikely in view of the nature of these sources of error, and it is therefore considered reasonable to include them in the over-all indeterminate error implied by the statistical analysis of the data.

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RECEIVED for review December 7, 1955. Accepted April 23, 1956. Division of Analytical Chemistry, 129th Meeting, ACS, Dallas, Tex., April 1956.

Micromethod for Determining Viscosity of High Viscosity Materials

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In studying the durability of asphalts by exposing thin asphalt films to the atmosphere, viscosity determinations on minute quantities of material are necessary. For that purpose a microviscometer has been developed which requires only 12 to 30 mg. of asphalt. The method is based on simple shear of the substance between two parallel plates and can be used for the determination of the viscosity of thermoplastic materials in the range of 10^4 to 10^9 poises with an accuracy within 5%. The microviscometer is suitable for investigations in which it is essential to work with small quantities of material and is a valuable aid in the analysis of asphalts recovered from bituminous constructions, such as road carpets. The method may also offer advantages in the study of fractionating processes, as viscosity measurements may be made on small fractions.

FOR the determination of the viscosity of highly viscous materials a micromethod has been developed, based on the principle of simple shear of the material between two parallel planes under the action of a constant shearing stress. Several other methods founded on the same principle have been published (*1*, *3*, *5*), but that described here differs from the others in requiring only very small amounts of material.

CONSTRUCTION

The microviscometer consists of two polished glass plates, both measuring 20 × 30 × 7 mm. Between these plates a thin layer of the substance under investigation is applied; the thickness of this layer may vary from 20 to 50 microns, so that a measurement of the viscosity requires only 12 to 30 mg. of material.

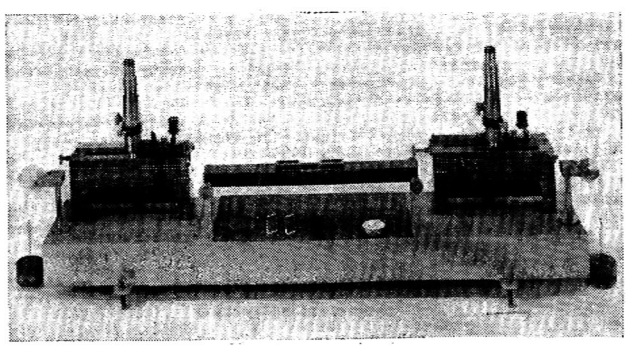

Figure 1. General view of microviscometer

The two glass plates are arranged in a horizontal position. The lower plate is fixed in a metal support; the upper plate is enclosed by a small frame, to which a weight is attached via a flexible steel wire running over a pulley. The wire carries a scale with divisions of 0.02 mm. The whole device is placed in an air thermostat, in which the temperature can be kept constant to within 0.1° C. between 25° and 40° C. Representations of a double apparatus without thermostat are given in Figures 1 and 2.

OPERATING PRINCIPLE

Under the influence of weight P a horizontally directed force is exerted on the upper glass plate so that shear takes place in the layer of the material under investigation. The displacement of the upper plate is read from the scale by means of a microscope. The viscosity can then be calculated immediately from the formula defining the viscosity of Newtonian liquids:

$$\tau = \eta \frac{d\gamma}{dt} \qquad (1)$$

where τ = shearing stress, dynes per sq. cm.
η = viscosity, poises
γ = shear = tangent of angle of shear
t = time, seconds

For calculating the viscosity, using the dimensions of the microviscometer, the formula employed is as shown in Equation 2.

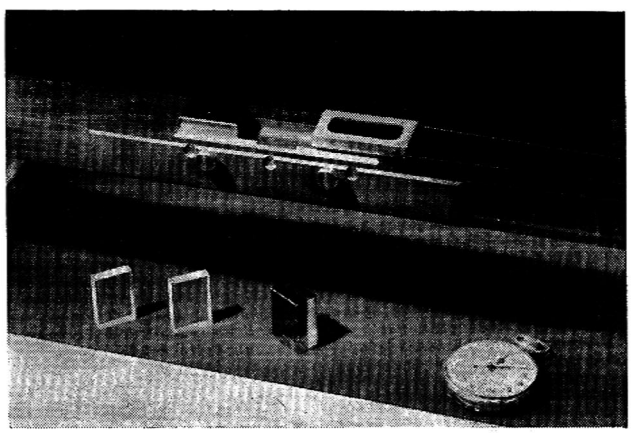

Figure 2. Measuring equipment for microscope reading

$$\eta = \frac{Pgr}{O} \frac{1}{ds/dt} \quad (2)$$

where P = load, grams
g = constant of gravitation
O = area of a glass plate, sq. cm.
s = displacement of upper plate, cm.
r = thickness of the material layer, cm.

With a constant rate of displacement v cm. per second of the upper plate:

$$\eta = \frac{Pgr}{vO} \quad (3)$$

To obtain a constant shearing stress during the measurement of the viscosity, the total displacement of the upper glass plate has to be limited to about 5% of the length of the plates—therefore, in this case to a displacement of 1.5 mm.

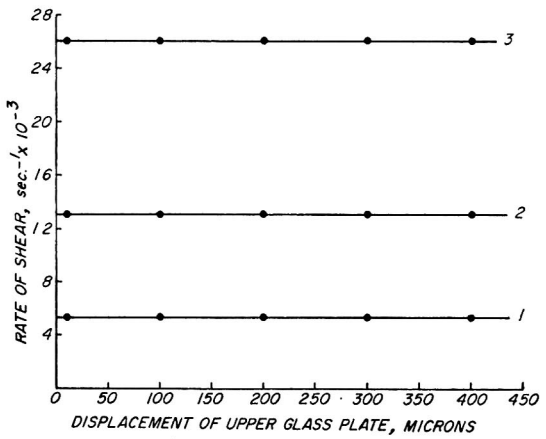

Figure 3. Relation between rate of shear and deformation at different shearing stresses for an Indonesian asphalt at 25° C.

Layer thickness, 24.5 microns
1. Shearing stress = 1.63 × 10^4 dynes per sq. cm.
2. Shearing stress = 4.08 × 10^4 dynes per sq. cm.
3. Shearing stress = 8.15 × 10^4 dynes per sq. cm.

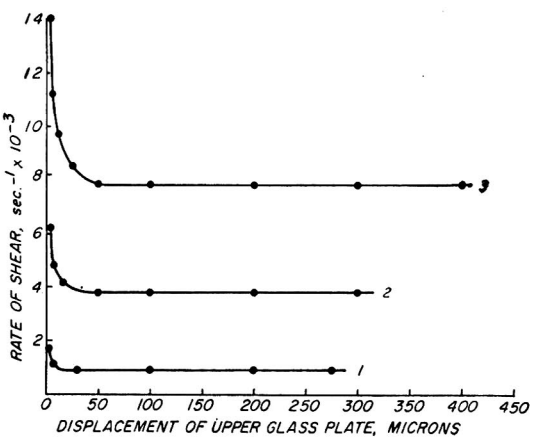

Figure 4. Relation between rate of shear and deformation at different shearing stresses for a Venezuelan road asphalt at 25° C.

Layer thickness, 30 microns
1. Shearing stress = 0.65 × 10^4 dynes per sq. cm.
2. Shearing stress = 2.70 × 10^4 dynes per sq. cm.
3. Shearing stress = 5.40 × 10^4 dynes per sq. cm.

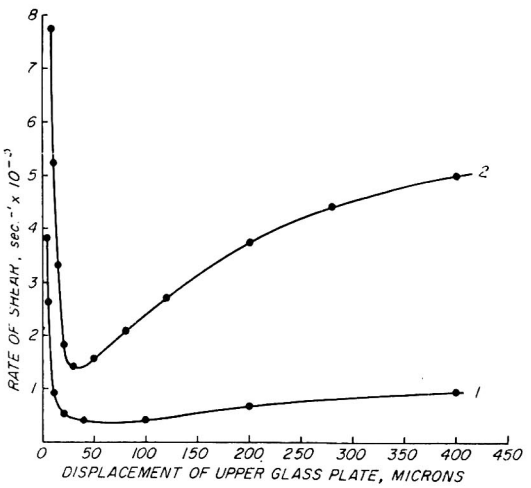

Figure 5. Relation between rate of shear and deformation at different shearing stresses for a Venezuelan blown asphalt at 35° C.

Layer thickness, 40 microns
1. Shearing stress = 2.90 × 10^4 dynes per sq. cm.
2. Shearing stress = 5.80 × 10^4 dynes per sq. cm.

The thin layer of material between the glass plates must be applied in such a way that good adhesion of the material to the glass is ensured. This is easily realized by applying a suitable quantity of the material dropwise to one of the glass plates at a temperature at which the material is sufficiently fluid and placing the second plate, also at that temperature, on the first and gently pressing until a homogeneous layer of the material has been obtained. Uniformity of thickness is controlled in transmitted light. After the sides of the glass plates have been cleaned, the thickness of the layer is determined by weighing.

SCOPE FOR APPLICATION

The viscosity range covered by the viscometer is 10^4 to 10^9 poises. In measuring materials showing Newtonian flow, a constant rate of shear is obtained throughout the measurement. Figure 3 shows measurements on an Indonesian asphalt with softening point ring and ball = 50.5° C.; penetration 25° C. = 50; penetration index = −2.1 (*10*).

Materials that possess viscoelastic properties show, under constant shearing stress, a deviation from constant flow at the beginning of the determination (*8*). In that region the rate of shear is higher and decreases to a constant value at greater deformations. Figure 4 gives an example of the rate of shear as a function of the displacement of the upper glass plate for a normal Venezuelan asphalt (softening point R&B = 55° C.; penetration 25° C. = 44; P.I. = −0.3) as used in road building. In this case constant rate of shear is obtained after a displacement of at most 50 microns. The viscosity of the asphalt is calculated from the horizontal parts of the curves. An influence of the shearing stress on the viscosity can be determined by measurements with different loads, as is also illustrated by Figure 4. In this case the rate of shear is almost proportional to the shearing stress applied.

The rheological properties of materials that on deformation do not show a constant rate of shear cannot be expressed by a single viscosity figure. Although the microviscometer was not intended for measurements on this type of materials, information on the rheological behavior of these substances can also be obtained with the instrument. In this case the relation between rate of shear, time or deformation, and shearing stress has

to be determined. An example is given in Figures 5 and 6 for a Venezuelan blown asphalt (softening point R&B = 68° C.; penetration 25° C. = 64; P.I. = +3.2).

In Figure 5, showing the relation between rate of shear and deformation, the high rate in the left part of the curves is due to the elastic properties of the asphalt, while to the right of the minimum the curves illustrate thixotropic phenomena caused by breakdown of structure in the material. The influence of the shearing stress on the rate of shear is highly dependent on the loading time, as illustrated by Figure 6. The deformations after 10 and 100 seconds (curve 1 and 2) are mainly elastic deformations.

Curves as given in Figures 5 and 6 can easily be obtained with the aid of an electrical recorder.

PRECISION AND ACCURACY

The reproducibility of the measurements was controlled by viscosity determinations on Indonesian asphalts of the Newtonian type. Measurements were carried out with layers of various thickness and various loads. The results are recorded in Table I.

The maximum deviation from the average values was 3 and 7%, respectively.

The absolute value of the viscosity was checked by determining the viscosity of an Indonesian asphalt, with a penetration at 25° C. of 133, by various methods. The results are given in Table II. The maximum deviation between the various methods is about 5%.

ELECTRICAL RECORDING

Electrical recording of the displacement of the upper glass plate offers advantages. Electrical recording can be executed in different ways, such as by means of dielectric or inductive devices (*4*), but these methods are somewhat complicated. A simple method, especially suitable for routine investigations under specified conditions, has been employed in this laboratory.

The construction of the apparatus is modified in such a way that a thin rod, *a*, is connected to the frame enclosing the upper glass plate (Figure 7). The other end of the rod is supported by a vertically mounted flexible blade, *b*, of about 0.25-mm. thickness. A second blade, *c*, is connected to the rod, which is provided with a pair of strain gages forming part of a bridge circuit. A displacement of the rod causes a slight bending of the strain gages; the corresponding change in the resistance of the bridge is indicated by a recorder.

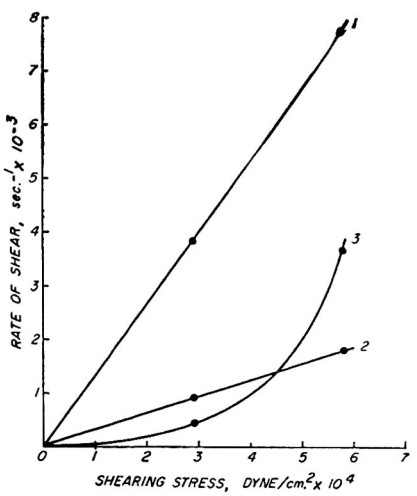

Figure 6. Relation between rate of shear and shearing stress at different loading times for a Venezuelan blown asphalt at 35° C.

Layer thickness, 40 microns
1. Loading time = 10 seconds
2. Loading time = 100 seconds
3. Loading time = 2000 seconds

Table I. Viscosity Measurements on Indonesian Asphalts with Microviscometer under Various Conditions, at 25° C.

Area of Glass Plate, Sq. Cm.	Thickness of Layer, Cm. × 10^3	Load, Grams	Shearing Stress Dynes/Sq. Cm. × 10^{-4}	Rate of Shear, Sec.$^{-1}$ × 10^2	Viscosity, Poises × 10^{-6}
Indonesian asphalt of penetration 98 at 25° C. (5 seconds)					
6	2.87	1000	16.3	19.0	0.86
6	3.05	500	8.15	10.0	0.82
6	3.68	250	4.08	4.9	0.84
6	3.68	100	1.63	1.9	0.86
Indonesian asphalt of penetration 50 at 25° C. (5 seconds)					
6	2.45	500	8.15	2.64	3.1
6	8.37	500	8.15	2.39	3.4
6	2.45	250	4.08	1.29	3.2
8	29.8	200	2.45	0.71	3.5
6	2.45	100	1.63	0.53	3.1

Table II. Viscosity Values of Indonesian Asphalt Determined by Various Methods

Method	Viscosity, Poises × 10^{-6}
Microviscometer	0.40
Calculated from penetration test (*9*)	0.40
Rotation viscometer (*2*)	0.38

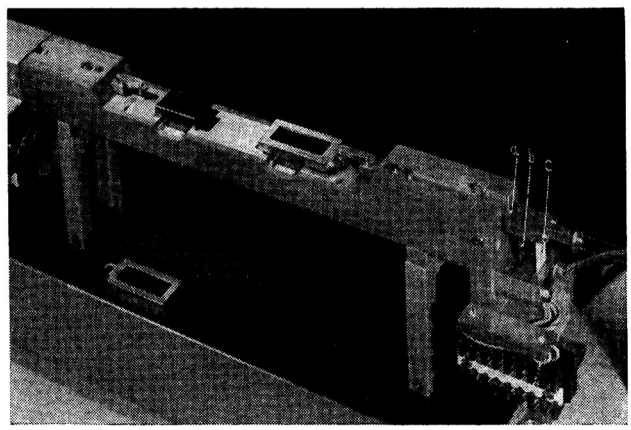

Figure 7. Measuring equipment for electrical recording

As the bending of blades *b* and *c* counteracts the applied force on the upper plate, the use of this method is limited; the displacement has to be kept small and the load fairly high. This device was constructed in such a way that on application of a load of 1000 grams and a maximum displacement of 200 microns, the decrease in shearing stress during the measurement is at most 3% of the original value. The method has been used in the investigation into the change in rheological properties of asphalts upon exposure to the atmosphere, as described later. In measurements carried out under other conditions than those mentioned above, another correction factor for the change in shearing stress has to be applied.

USE OF MICROVISCOMETER

General. The microviscometer has been used in the study of the hardening of asphalts by exposure to atmospheric conditions (*6*).

Table III. Thickness of Layers in Relation to Rate at Which Glass Plate Is Drawn from Solution

Diameter of Pulley on Driving Shaft, Cm.	Circumferential Velocity[a], Cm./Min.	Thickness of Asphalt Layer, Microns
1	3.14	4.2
2	6.28	6.3
3	9.42	8.4

[a] Rate at which plate is drawn upward.

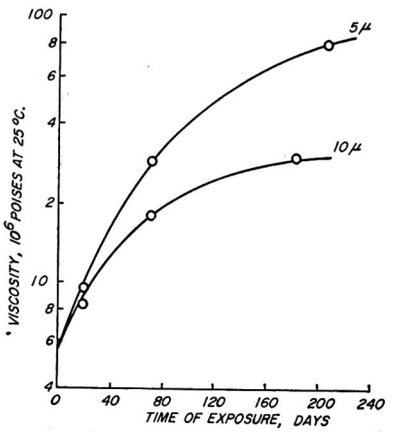

Figure 8. Increase in viscosity of Venezuelan asphalt 50/60 penetration on exposure to air at room temperature with exclusion of light in layers of 5- to 10-micron thickness

Shearing stress = 1.63 × 10^5 dynes per sq. cm.

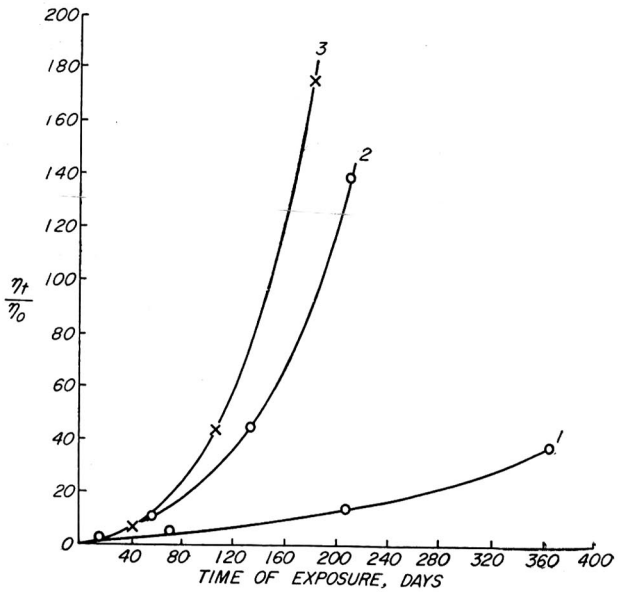

Figure 9. Hardening of Venezuelan asphalt 50/60 penetration on exposure to various atmospheric conditions in layers of 5-micron thickness as a function of time of exposure

1. Room temperature, darkness
2. Room temperature, diffused daylight
3. 40° C., darkness

In the practical application of asphaltic constructions in which the asphalt is exposed to the influence of light and air, changes in the mechanical properties of the asphalt are observed. These changes are restricted to a surface layer in the asphalt a few microns thick. For the study of the hardening effects it is therefore necessary to expose very thin layers of asphalt to the atmosphere; if thicker layers are used, the greater part of the asphalt remains unaltered, so that the hardening of the top layer results in only a very small change in properties of the total quantity of asphalt.

The technique followed in the present investigation therefore consists in exposing asphalt films of 5 to 10 micron thickness on glass plates to the influence of the atmosphere. Subsequently the aged films are scraped off the plates and the viscosity of the material is determined with the aid of the microviscometer.

Preparation of Asphalt Films on Glass Plates. The application of thin asphalt films to glass plates was carried out according to the method of Payne (*7*). A glass plate, 10 × 10 cm., is immersed in a solution of the asphalt in benzene, concentration about 50 weight %, and drawn from the solution at a constant rate with the aid of a synchronous motor. If the apparatus is so arranged as to be free from vibration, the procedure yields a homogeneous asphalt film, the thickness of which is a function of the rate at which the glass plate is drawn from the solution. This rate can be varied by using pulleys of different diameters on the driving shaft of the motor around which the cord attached to the plate is wound. Examples are given in Table III.

After a drying period of 1 to 2 hours, the benzene has completely evaporated; the thickness of the asphalt layer is calculated from the increase in weight of the glass plate, the area covered, and the specific gravity of the asphalt.

Oxygen and light must be excluded in preparing the asphalt films. The preparation of the asphalt-benzene solution, the drawing of the plates from the solution, and the drying should take place in a nitrogen atmosphere and in the dark or in red light. If these precautions are not taken, a considerable hardening of the asphalt will take place in making the films. Nevertheless, even with these precautions, some hardening is unavoidable; the increase in viscosity may sometimes amount to as much as 30%. However, the effect of the exposure is much greater, so the undesired hardening during preparation can be eliminated by taking as the original viscosity that of the asphalt on an unexposed plate.

The asphalt-covered plates are exposed to the atmosphere, the asphalt is then scraped off the plate with a razor blade, and its viscosity is determined by means of the microviscometer.

Hardening of Asphalt Exposed to Atmosphere. A few results obtained with the microviscometer in the study of the hardening of asphalt are given in Figures 8 and 9. Figure 8 shows the effect of the layer thickness on the change in viscosity of a Venezuelan asphalt with a penetration of between 50 and 60 at 25° C. when exposed to the air with exclusion of light for about 6 months.

Figure 9 represents the change in viscosity of the same asphalt when exposed to different atmospheric conditions in layers of 5 micron thickness. The change in viscosity is here expressed in the ratio between the viscosity after exposure, η_t, and the original viscosity, η_0, as a function of the time of exposure. The viscosity measurements were carried out with a shearing stress of 1.63 × 10^5 dynes per sq. cm.

ACKNOWLEDGMENT

The authors wish to thank the management of the Koninklijke/Shell-Laboratorium, Amsterdam, for permission to publish this paper.

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RECEIVED for review September 9, 1954. Accepted February 20, 1956. Division of Petroleum Chemistry, 126th Meeting, ACS, New York, September 1954.

General Photometric Microdetermination of Cobalt with Nitroso-R Salt

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A photometric method is presented, which makes possible determination of cobalt at 425 mμ by the use of potassium bromate with a fivefold increase of sensitivity over procedures using 525 mμ, and introduces midanalytical evaluations that free the analyst from the responsibility of knowing the approximate concentration of cobalt in the sample. If magnesium acetate is used, large amounts of fluorides can be added to remove interferences without fear of etching the absorption cells. The procedure has been evaluated by tracer techniques using $_{27}Co^{60}$.

SINCE 1921, when Van Klooster (*8*) introduced the use of nitroso-R salt as a reagent for cobalt, there has been considerable difference of opinion as to the best wave length for the measurement of the absorbance of the cobalt–nitroso-R salt chelate. Willard and Kaufman (*9*) studied the absorption spectrum of cobalt–nitroso-R salt and concluded that 420 mμ was the optimum wave length for analysis. Others (*2*, *3*, *5–7*) have preferred 510, 525, or 550 mμ for measuring the absorption of the chelate.

A study of the absorption characteristics of both the chelate of cobalt with nitroso-R salt and the nitroso-R salt indicated that quantitative absorption measurements can be made at 525 and 425 mμ. Measuring the absorbance of the cobalt chelate at 525 mμ makes possible a relatively wide range of cobalt concentration with moderate sensitivity, while the 425 mμ wave length increases the sensitivity with a narrower range. At these wave lengths there also is exhibited a widely differing reagent absorption; it is most severe at 425 mμ. To eliminate this Marston and Dewey (*4*) used bromine to decolorize the excess reagent. Experience indicates that the reaction is very sensitive to the bromine concentration and large errors can result from a slight excess of bromine. Other investigators (*1*) have used chlorine with some increase in stability. To simplify the manipulations and increase the stability, other oxidizing agents were investigated; potassium bromate was the most satisfactory.

The method submitted here may be adapted to the determination of cobalt in steels, soils, and water after samples have been dissolved by appropriate methods.

The cobalt concentration in the final working volume must be brought to within the range of 0.01 to 2.8 p.p.m.

REAGENTS AND APPARATUS

33% sodium acetate, C.P.
33% potassium fluoride, C.P.
3% potassium bromate, C.P.
30% magnesium acetate tetrahydrate, C.P.
Barium acetate, C.P.
Concentrated nitric acid, specific gravity 1.410, C.P.
Acetic acid, glacial, C.P.
Nitroso-R salt, 0.5 gram per 100 ml.
Bromine, liquid, C.P.
Filter photoelectric photometer
Beckman Model DU quartz spectrophotometer

PROCEDURE

To attain the maximum color of the chelate and the optimum concentration, the following conditions should be met.

The pH should be 5.5 ± 0.5; this is accomplished by use of acetate–acetic acid buffer medium.

If the solution is to be evaporated to 5 ml. for increased sensitivity, the sulfate ion, if the sample is fused, and the chloride ion, if present, should be removed. Use of barium acetate and silver nitrate is recommended for the removal of these ions. If the sample has been fused in potassium pyrosulfate, no chloride ion is present. If both reagents are used, there must not be an excess of both or the nitroso-R salt will precipitate.

Interferences must be removed. For this purpose potassium fluoride is used.

This procedure makes possible an analysis when the chemist has but one small sample. The procedure embodies several intermediate tests that lead to differing subsequent steps.

Remove Interferences. If iron is present, oxidize it with bromine and boil to expel the excess before the addition of 1 ml. of 33% potassium fluoride.

Remove chloride ions with silver nitrate after acidification with glacial acetic acid.

Remove sulfate ions by the addition of barium acetate.

Remove fluoride ions, in excess of the amount needed to complex or precipitate interferences, with magnesium acetate.

Determine Range. If the sample is a water solution (which has received no treatment), add 1 ml. of sodium acetate and 0.1 ml. of glacial acetic acid. Add approximately 85 μl. of 0.5% nitroso-R salt and visually estimate the amount of cobalt (by comparing with standards).

If less than 5 γ of cobalt is present, remove chloride ion and sulfate ion as outlined above and continue procedure.

If more than 5 γ of cobalt is present, add 1 ml. of nitroso-R salt, heat to boiling, and add 1 ml. of concentrated nitric acid; cool, dilute to 25 ml., and determine absorbance at 525 mμ against a reagent blank.

If the sample is steel, after removing iron as outlined above add 2 ml. of 30% solution of magnesium acetate and centrifuge to remove magnesium fluoride. Adjust the pH of the clear super-

natant liquid to 5.5 ± 0.5, add 85 μl. of 0.5% nitroso-R salt, and visually estimate the cobalt content.

If less than 5 γ of cobalt is present, remove sulfate ions as outlined above and continue procedure.

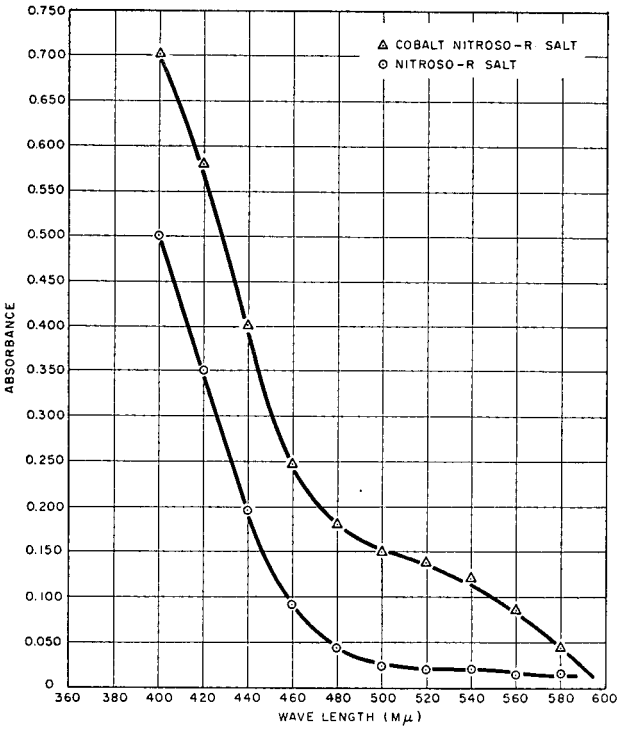

Figure 1. Absorption spectra of nitroso-R salt and cobalt–nitroso-R salt complex

If more than 5 γ of cobalt is present, add 1 ml. of nitroso-R salt, heat to boiling, add 1 ml. of concentrated nitric acid, dilute to 25 ml., and measure the absorbance at 525 mμ against a reagent blank.

If the sample is a cobalt oxide that has been fused to make it soluble, add 1 ml. of 33% sodium acetate, 0.1 ml. of glacial acetic acid, and 85 μl. of nitroso-R salt.

Estimate Cobalt Content. If less than 5 γ of cobalt is present, remove sulfate by addition of 1 gram of barium acetate and continue procedure.

If more than 5 γ of cobalt is present, add 1 ml. of nitroso-R salt, heat to boiling, add 1 ml. of concentrated nitric acid; cool, dilute to 25 ml., and measure the absorbance at 525 mμ against a blank.

Concentrate Sample to 3 ml. by evaporation.

Oxidize Excess Nitroso-R Salt by addition of 500 μl. of a 3% potassium bromate solution; heat to boiling and add 0.5 ml. of concentrated nitric acid. The pH should be approximately 1. Cool and dilute to 5 ml.

Measure Absorbance at 425 mμ against a water blank.

DISCUSSION AND RESULTS

The method presented has been used successfully in the microdetermination of cobalt in a large number of soil samples. The sensitivity has been increased by a factor of 5, in the range 0 to 5 γ of cobalt, over that of a previous procedure (*5*). The use of potassium bromate to oxidize the excess nitroso-R salt selectively, so as to leave the cobalt–nitroso-R salt chelate intact, is critically dependent upon the final nitric acid concentration. The elimination of chlorides is essential, as they combine with nitric acid to form aqua regia, which destroys the nitroso-R salt.

Two different wave lengths are used to measure the absorbance of the cobalt–nitroso-R salt chelate. As shown in Figure 1, at a given concentration the absorbance of the cobalt chelate at 425 mμ is much greater than at 525 mμ, but the absorbance of nitroso-R salt is negligible at 525 mμ and a considerable factor at 425 mμ. Therefore the excess nitroso-R salt is destroyed with potassium bromate when the absorbance is measured at 425 mμ.

An important question is: How much cobalt is lost during the recommended precipitations? To answer it, radioactive cobalt $_{27}Co^{60}$ was used and measurements were made in a 100% geometry gamma ionization chamber. To evaluate the loss by the precipitation of silver chloride, 7.16 μc. of cobalt-60 was added to a solution known to contain 6.2 γ of cobalt ion and 0.67 meq. of chloride ion. After the addition of silver nitrate and centrifuging, the activity in the residue was 0.0195 μc., indicating a loss of only 0.27%. A second check on soil was made using the tracer technique. A 200-mg. sample of soil, known to contain 2.91 γ of cobalt ion, was fused after the addition of 1.49 μc. of cobalt-60. In the sulfate precipitation 0.010 μc. of activity was found. The supernatant liquid after color development contained 1.37 μc. of cobalt-60, indicating the transfer and precipitation loss for the entire procedure to be approximately 0.12 μc. of tracer or 0.2 γ of cobalt ion.

Table I. Precision of Method

Mμ	No. of Detns.	Co Present, γ	Standard Deviations[a] %
425	4	0.44	2.27
	4	1.2	3.33
	4	2.4	1.25
	4	2.9	0.69
	4	4.5	1.11
525	4	5.0	No deviation
	6	10	2.17
	12	20	1.08
	10	30	1.13
	46	40	0.45
	12	50	0.66
	11	60	0.22
	12	70	0.47

[a] Calculated by following equation

$$\text{Sigma} = \sqrt{\frac{\Sigma(x - \bar{x})^2}{n}}$$

where x is a numerical result, $\bar{x}$ is the mean value of x, and n is the number of determinations.

Samples containing known amounts of cobalt were fused with cobalt-free soil and the amount of cobalt in each sample was determined by the procedure described above. The resulting working curves obeyed Beer's law over the concentration range 0.5 to 5 γ of cobalt per 5 ml. In the case of higher concentrations of cobalt, over the range of 5 to 70 γ per 25 ml., a working curve must be constructed, because curves over this range are slightly parabolic. The precision of the method is summarized in Table I.

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RECEIVED for review November 17, 1955. Accepted April 20, 1956.

Determination of Micro Quantities of Hydrogen

Combustion-Manometric Method

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A combustion method has been developed for the determination of micro amounts of hydrogen in uranium and other materials. The water produced from the combustion of the sample in an oxygen stream is collected in a capillary trap at −78° C. The trap, a U-tube which may be swiveled upward or downward by means of semiball joints, serves also as the fixed volume leg of a constant volume manometer. The vapor pressure of the entrapped water is then measured at 100° C. A low and stable blank with an average daily standard deviation of 0.02 γ of hydrogen permits the method to be sensitive to quantities of hydrogen less than 0.1 γ. Simultaneous microdeterminations of both hydrogen and carbon can be completed within a half hour. The sample is not subjected to low pressures.

INTEREST in the properties of high purity uranium as related to hydrogen content has created the need at this laboratory for a suitable method for the determination of hydrogen in the microgram to submicrogram-range. Methods that have been used by others for low-range hydrogen analyses include vacuum fusion (*1, 4, 6, 11*), warm extraction (*6*), and low-pressure combustion (*5*). A method employing a sensitive mercury-oil manometer with a modified Pregl apparatus has also been described (*9*) for microdeterminations of hydrogen and carbon in organic compounds.

Inasmuch as the capillary trap method (*7, 10*) is used at this laboratory for the determination of low carbon, it was particularly desirable to employ a complementary manometric method for the determination of micro quantities of hydrogen, so that the two elements might be measured simultaneously, if desired. Such a method is described here as it was applied to uranium, zirconium hydride, and some organic compounds, the samples of which contained amounts of hydrogen ranging from 0.04 to 30 γ.

The sample is burned in a stream of oxygen and the water produced in combustion is selectively frozen out and measured in a specially designed swivel-jointed constant volume manometer (*3*). High precision is achieved by heating the isolated water vapor to 100° C. before measuring its pressure. The time required for a complete microanalysis for both hydrogen and carbon is a half hour or less.

APPARATUS

Figure 1 is a schematic diagram of the apparatus showing the relative positions of the component parts. Laboratory space was conserved by placing the parts shown in the left half of the diagram behind those in the right half.

Both argon and oxygen were available for use in the combustion of uranium metal, which normally burns rapidly. Argon, flowing through the train at the time the sample was placed in the combustion tube, provided an inert atmosphere at the beginning of the combustion period. A switch to oxygen created in the purification system a gradient of gas mixtures becoming increasingly rich in oxygen. Under these conditions the uranium burned mildly and evenly, instead of violently as when ignited in pure oxygen. A slight positive gas pressure was maintained by the manostat which consisted of two 4-liter bottles. The manostat also provided extra gas when needed, such as when the combustion tube was opened, or when additional oxygen was required during sample combustion. The rate of flow was governed by the grooved stopcock, S_8, and was indicated by the carbon manometer. These controls for line pressure and flow rate were described earlier by Smiley (*10*). The flow rate used was about 125 ml. per minute.

Tank grade oxygen and argon were purified by passing them over heated copper oxide, followed by silica gel, Drierite, Ascarite, and magnesium perchlorate, as indicated in Figure 1. The fused quartz tube for the copper oxide was 16 inches by 26 mm.

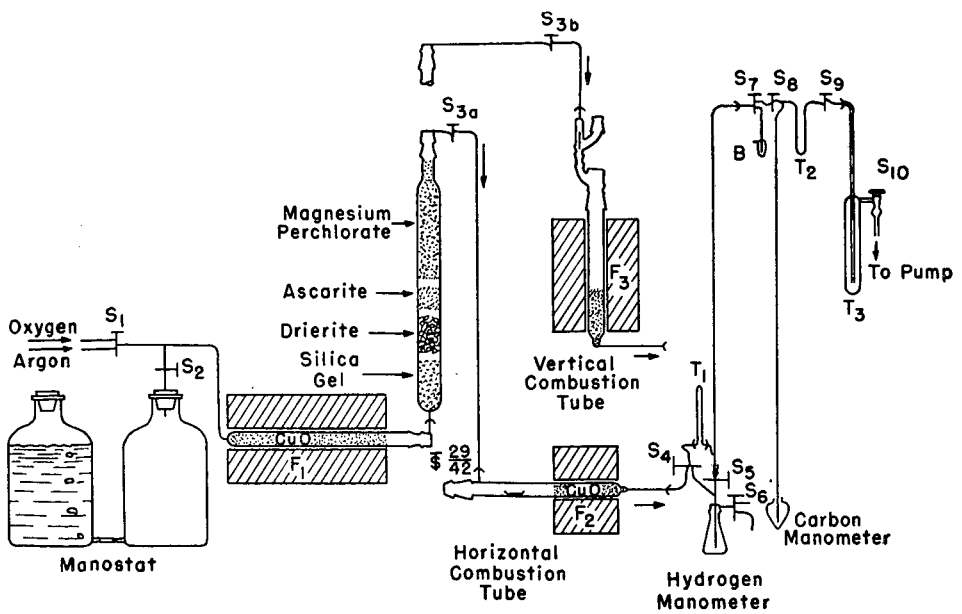

Figure 1. Schematic diagram of apparatus used for microdetermination of hydrogen and carbon

(outside diameter); the vertical borosilicate glass tube holding the other reagents was 20 inches by 46 mm.

Two types of combustion tubes were used, one horizontal and one vertical. The vertical tube, shown in more detail in Figure 2, gave lower blanks and a higher sensitivity, but was limited to chunk samples added via the electromagnetic sample dumper. The horizontal tube was more convenient for finely divided samples, but the use of the quartz sample boat was found to contribute substantially to higher and more variable blanks.

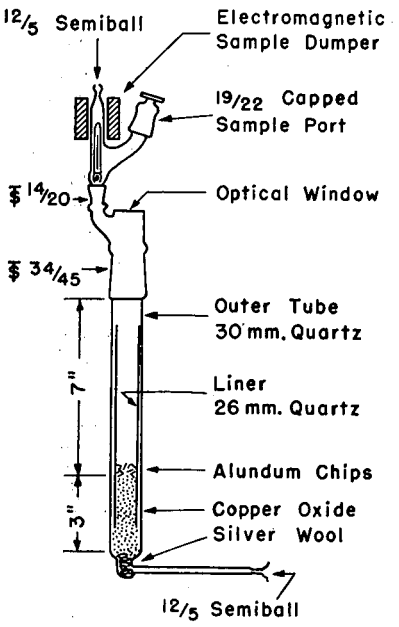

Figure 2. Vertical combustion tube

The horizontal combustion tube was of fused quartz, 13 inches by 25 mm. (outside diameter). Copper oxide, confined to the exit end of the tube by a scrap of platinum gauze, was maintained at 900° C. to ensure complete combustion. The glazed quartz boats were $3^1/_8$ inches long, $^3/_4$ inch wide, and $^3/_8$ inch deep. Heat for sample combustion was supplied by a Fisher burner operating at approximately 1000° C. Above the burner a $2^1/_2$-inch strip of platinum foil surrounded the combustion tube to provide even distribution of heat to the enclosed boat.

Beyond the copper oxide bed in each combustion tube was a small tuft of silver wool for chloride removal. Connection was made from the combustion tube to the hydrogen manometer by a 12/5 semiball joint sealed with Apiezon-T grease. The span of apparatus between the furnace, F_2, (or F_3), and stopcock, S_4, was maintained at 100° C. by means of a Variac-controlled, 2-foot heater tape to prevent adsorption of the water vapor on the glass walls while en route to the manometer.

A detailed sketch of the swivel-jointed constant volume manometer is shown in Figure 3. The capillary U-tube was designed to serve both as a cold trap for collection of the water vapor and as the fixed volume manometer leg which was heated to 100° C. for pressure measurement. This twofold role was made possible by the swivel arrangement provided by the two pairs of 12/2 semiball joints held firmly together by a wire coil spring. The glass wool plug was enclosed in the 7-mm. end section of the U-tube to serve as a filter for retention of ice crystals formed in the capillary during condensation. The other 7-mm. section was included for an alternate calibration range. Pressure-type stopcocks were used, and colored glass extensions were fused to the plug handles of the two three-way stopcocks for position identification. For needle-valve control, stopcock S_4 was taper-grooved for the position connecting the manometer to the combustion tube, and stopcock S_6 was taper-grooved for both the air and the vacuum positions. The manipulations involved in the use of the manometer are described in the procedure.

The carbon manometer (Figure 1) has been described by Smiley (*10*) and Holt (*7*). The liquid nitrogen trap, T_3, was used to protect the train from back-diffusion of pump oils and condensable gases. A Welch Duo-Seal pump provided vacuum for both the analytical train and the mercury reservoir.

An electric heat gun with a turbo-type blower was used to raise the temperature of the U-tube quickly to 100° ± 1° C. It was manufactured by the Master Appliance Co., Racine, Wis., and, when operated at 110 volts and 5 amperes, it had a recommended temperature range of 200° to 300° F. The equilibrium temperature at full heat was regulated by adjusting the air intake. A glass T-joint, indicated in Figure 4, *c*, was fabricated to serve as the hot-air flue surrounding the U-tube and as the coupling to the barrel of the gun. An asbestos disk flange affixed to the lower end of the U-tube (upright position) prevented the hot air from emerging from the lower end of the T-joint.

Furnaces F_1, F_2, and F_3 (Figure 1) were, respectively, 12-inch, 4-inch, and 8-inch multiple units, manufactured by the Hevi Duty Electric Co., Milwaukee, Wis. The operating temperatures were 900° C. for F_1 and F_2, and 1000° C. for F_3.

PROCEDURE

A series of schematic diagrams in Figure 4 illustrates the manipulations involved in the use of the swivel-jointed manometer. Figure 4, *a*, shows the arrangement used during sample combustion.

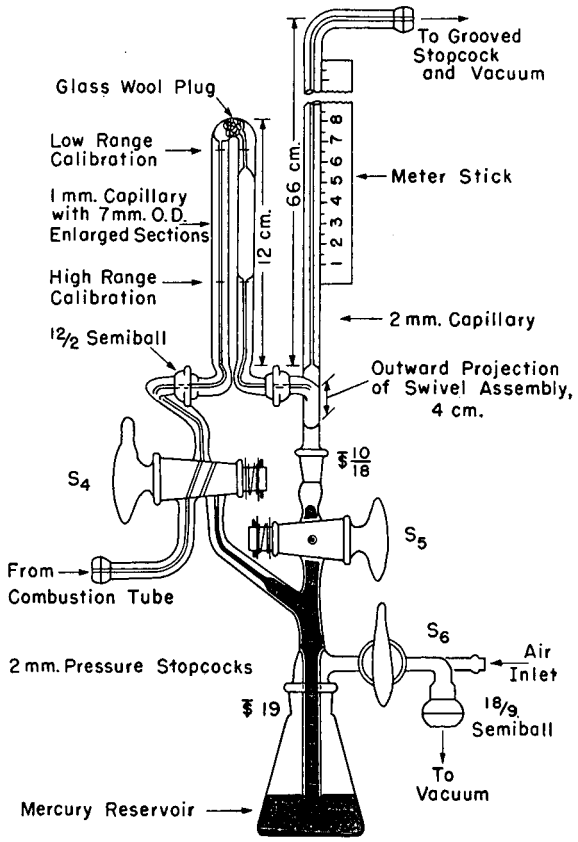

Figure 3. Swivel-jointed manometer

Water vapor is condensed from the oxygen stream in the U-tube, T_1, and cooled by dry ice and acetone, while carbon dioxide is carried through to the carbon manometer where it is condensed out with liquid nitrogen in T_2 (Figure 1). During this operation oxygen is replacing argon (if used) throughout the train, with a slight excess escaping through the manostat; and the grooved stopcock, S_8, is controlling the flow rate at about 125 ml. per minute. The pressure in T_1 is slightly above atmospheric while that in T_2 is about 50 mm. of mercury, which is low enough to prevent condensation of either oxygen or argon with the carbon dioxide.

At the end of a 10-minute combustion period stopcock S_4 is closed and S_8 (Figure 1) is completely opened to evacuate both manometers. When the pressure reading is zero on the carbon manometer, stopcocks S_4 and S_5 are turned to connect the hydrogen manometer to the mercury reservoir. When air is admitted to the partially evacuated reservoir through S_6, the mercury is

driven upward into the three legs, thus entrapping any water vapor frozen out in the U-tube. Then, as indicated in Figure 4, *b*, the U-tube is swiveled upward and the mercury is driven farther upward past the joints to a level which can be included in the zone heated by the heat gun. The heat gun is placed in the indicated position; a 3-minute period suffices to bring the temperature of the U-tube to 100° ± 1° C.

It is during this heating period that the carbon measurement is made, if desired, by the following steps: Close stopcocks S_8 and S_9; remove the liquid nitrogen fron T_2; warm to room temperature and observe the depression of the mercury column. A calibration curve relating depression to micrograms of carbon gives the carbon content. Stopcocks S_8 and S_9 are reopened.

At the end of the 3-minute heating period air is again admitted to the mercury reservoir to drive the mercury to the top calibration mark on the U-tube, Figures 3 and 4, *c*. Because of the concombustion tube. The train is now prepared either to make another analytical run or to close down, as for overnight.

To make another run using the horizontal combustion tube, the oxide of the previous sample is discarded and the boat is quickly returned to the mouth of the tube where the effluent purified gas, supplied by the manostat, protects it from further contamination by atmospheric moisture. The next sample is placed in the boat and it is returned to its position over the burner. The cap of the combustion tube is replaced but not snugly fitted for a few seconds to allow the entrapped air to be flushed away. The U-tube, T_1, is turned downward and about 2 inches of the lower end is submerged in the mixture of dry ice and acetone. The capillary trap, T_2, is immersed in liquid nitrogen and stopcock S_8 is adjusted as before to give a flow of 125 ml. per minute. The burner is turned on; stopcock S_1 is turned from argon to oxygen; and an alarm timer is set for 10 minutes.

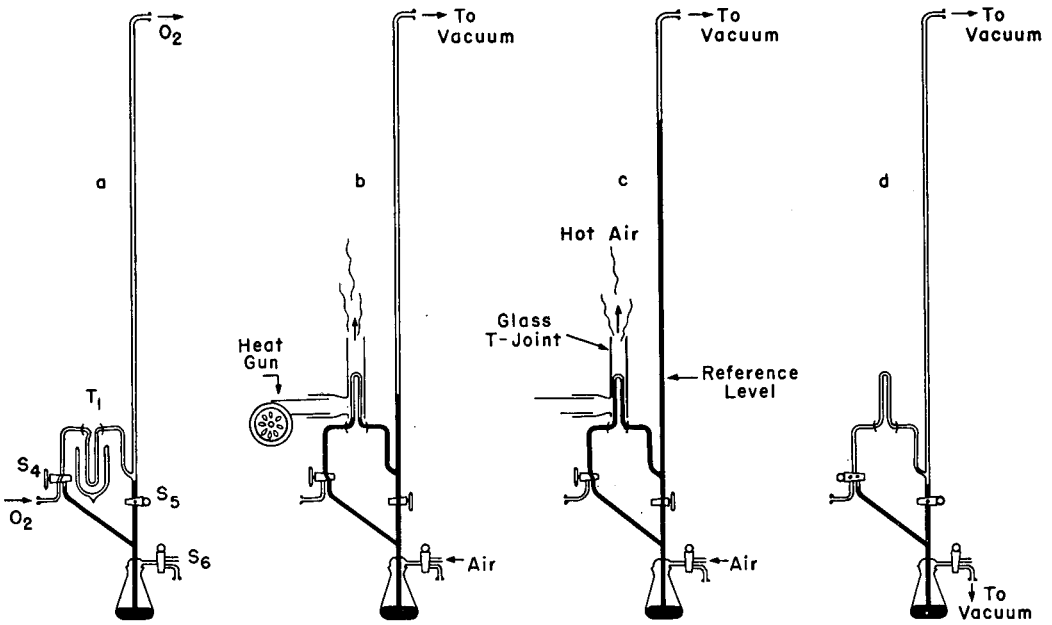

Figure 4. Manipulations of swivel-jointed manometer during run

a. Condensation of water at −78° C.
b. Evaporation of water with heat gun
c. Manometer reading of water vapor pressure at 100° C.
d. Discharge of water preparatory to next run

finement of the water vapor to the small fixed volume, the mercury in the other leg of the manometer is driven up above the reference level to a height which is proportional to the amount of steam confined, and hence to the amount of hydrogen originally present in the sample. If the amount of confined water vapor is so great that the mercury cannot be raised to the top calibration mark, even when the mercury reservoir is subjected to atmospheric pressure, the mercury is lowered to the high-range calibration marks (Figure 3) for measurement. The manometer used in this work had a low-range fixed volume of 0.225 ml., affording sensitive measurement of 0.00 to 7.00 γ of hydrogen, and a high-range volume of 1.47 ml., measuring 0.0 to 60 γ of hydrogen.

After the measurement is completed, the heating element of the gun is cut off and unheated air is allowed to blow through the flue for about 1 minute to cool the U-tube. Care must be taken to maintain stable conditions by lowering the mercury in the measuring column as the water vapor recondenses. After the U-tube is cooled to approximately room temperature, the gun is removed and the remaining water vapor pressure is essentially eliminated by cautiously painting the bend with dry ice–acetone slush. The mercury is lowered to the original position and stopcocks S_4 and S_5 are closed (Figure 4, *d*). To void the apparatus completely of moisture in preparation for the next run, stopcock S_4 is opened slightly to the combustion tube until the gas flow through the line is such as to effect a depression of the mercury in the carbon manometer of about 10 cm. The U-tube is now flamed to ensure thorough degassing of the glass wool plug. (If the horizontal combustion tube is being used, the gas supply is changed from oxygen to argon at S_1.) This low-pressure flushing is continued for 2 minutes, after which S_8 is closed and S_5 is opened completely

If the vertical tube is used, the sample is simply placed in the sample dumper where it remains until the entry port is closed; traps T_1 and T_2 are immersed in coolants; and the flow is adjusted as described above. The violent combustion reaction of uranium is not as objectionable in the vertical tube as in a combustion boat in a horizontal tube; hence, the use of argon may be eliminated, especially with small samples.

To close the line down rather than to prepare for another run, S_7 is opened to the bubbler, *B;* S_2 is closed; and S_1 is connected to oxygen. The flow through the bubbler is adjusted to about 1 bubble per second with a needle valve on the oxygen supply, and S_9 and S_{10} are closed before shutting off the vacuum pump.

DISCUSSION

No oxides of nitrogen were expected to be produced by the combustion of any of the materials analyzed; consequently, no provision was made for the removal of such. According to Johns (*8*) uranium nitride burns to form a uranium oxide and nitrogen, but no nitrogen oxides.

The density change of the mercury in the heated U-tube, T_1, had a negligible effect upon the manometer reading. The reading was the same, ±1 mm., whether the tube was nearly filled with mercury while heating to 100° C., or whether the mercury was freshly supplied from the unheated reservoir just before taking a reading.

There was no evidence of grease accumulation in the U-tube

of the hydrogen manometer. The mercury passing through the greased semiball joints transported some "dirt" downward but none upward into the U-tube.

CALIBRATION

The hydrogen manometer was calibrated by measuring the pressures of the water vapor produced when known quantities of hydrogen gas were burned in the combustion tube. A dosing stopcock similar to that described by Brown (*2*) was inserted into the line between the purification tube and the combustion tube. The volumes of the two bores ground into the stopcock plug were 0.0097 and 0.0884 ml. By using the two bores in multiple doses a range of sample sizes was made available for checking the calibration along all portions of the manometer scale. Tank hydrogen was used, each dose being taken at barometric pressure and room temperature. The calibration factor, or the average ratio of hydrogen weight to manometer reading, for the low-range mark was 0.0195 γ of hydrogen per mm., with a standard deviation of 0.0002 γ of hydrogen per mm. for seven determinations. The corresponding factor for the high-range mark was 0.1272 ± 0.0011 γ of hydrogen per mm. for twelve determinations. Each factor was constant over its entire range.

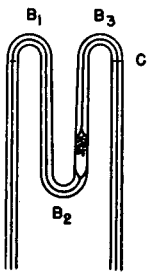

Figure 5. Earlier design of manometer trap

EXPERIMENTAL DATA

During the process of developing the method two modifications were made in the design of the apparatus, both of which improved sensitivity. The fixed volume of the manometer was considerably reduced and the blank for a complete analytical run was made lower and more stable. As indicated in the tables, some of the reported data were gathered before and some after these improvements.

The earlier and less sensitive design of the fixed-volume portion of the manometer is shown in Figure 5. Bend B_2 was submerged in the coolant for condensation, whereas for heating, all three bends, B_1, B_2, and B_3, were enveloped in the heating unit. B_1 and B_3 were constructed perpendicular to B_2 for compactness. To make a measurement of the heated water vapor, the mercury was driven up to the calibration marks, C, which were the limits of volume reduction without losing control of the mercury. The change-over to the swivel-jointed trap reduced the space of gas confinement by a factor of 2.6, with a corresponding increase in sensitivity. The data shown in Tables III and IV were obtained on the early, less sensitive manometer.

The blank for a complete analytical run was lowered and stabilized by changing from the quartz horizontal to the quartz vertical combustion tube. Table I shows comparative data for the two tubes. The vertical quartz tube had a lower average blank with a much narrower range of values. The variation within a single day of operation was also greatly reduced. Table II further displays this stability. The necessary handling of the quartz sample boat with the horizontal tube is doubtless responsible for the blank differences. The amount of water which it absorbs during its brief exposure to the air is apparently significant when analyzing for hydrogen at such low levels.

Table I. Comparison of Blanks Obtained with Three Types of Combustion Tubes

Type of Combustion Tube	No. of Blanks	Hydrogen, γ Range	Hydrogen, γ Av.	Hydrogen, γ Av. spread per day (highest − lowest)
Horizontal, quartz	41	0.04 to 0.60	0.25	0.20
Vertical, quartz	28	0.02 to 0.18	0.09	0.06
Vertical, sillimanite	23	0.25 to 1.56	0.77	0.38

Table II. Typical Daily Blank Variation Using Vertical Quartz Combustion Tube

Day	No. of Blanks	Hydrogen, γ Mean	Hydrogen, γ Std. dev. from mean
1	3	0.04	±0.02
2	5	0.04	±0.02
3	4	0.06	±0.02
4	3	0.10	±0.04
5	6	0.14	±0.02
6	4	0.14	±0.02
		Av. std. dev. from mean	±0.02

Table III. Hydrogen Recovery Data on Methane, Hydrogen Gas, and Sucrose

(Horizontal tube and less sensitive manometer used)

Material	Hydrogen, γ Added	Hydrogen, γ Found	Hydrogen, γ Dev.
Methane	1.4	1.4	0.0
	1.4	1.3	−0.1
	2.7	2.8	+0.1
	2.8	2.8	0.0
	1.4	1.4	0.0
	14.0	13.9	−0.1
	13.9	14.0	+0.1
	13.9	13.7	−0.2
	13.9	13.6	−0.3
	13.9	13.9	0.0
	13.9	13.9	0.0
	13.9	14.0	+0.1
	27.8	28.2	+0.4
	27.8	28.1	+0.3
	27.8	27.9	+0.1
		Av.	±0.1
Hydrogen	0.68	0.61	−0.07
	0.67	0.61	−0.06
	0.33	0.35	+0.02
	0.50	0.51	+0.01
	0.21	0.20	−0.01
		Av.	±0.03
Sucrose	0.2	0.3	+0.1
	0.2	0.1	−0.1
	0.4	0.5	+0.1
	0.4	0.4	0.0
	0.6	0.4	−0.2
	0.6	0.9	+0.3
	0.8	0.5	−0.3
	0.8	1.0	+0.2
	1.0	0.7	−0.3
	1.0	0.9	−0.1
	1.2	1.5	+0.3
	1.4	1.0	−0.4
	1.6	1.4	−0.2
		Av.	±0.2

Table I also contains comparative blank data for a vertical sillimanite McDaniel tube, showing that it was inferior to either of the other two. Attempts were made to utilize this highly refractory material because a quartz tube wall, heated to 1000° C. by the surrounding furnace, had been found to fail when subjected to the additional heat generated by a rapidly burning 1-gram sample of uranium. This difficulty was overcome, however, by including the quartz liner shown in Figure 2. The attacks by heat and slag formation are spent on the liner, leaving the outside tube walls unharmed.

It is possible that the increased sensitivity gains may be offset by other interfering factors in the analysis of a given material. The sample may contain a heterogeneous distribution of metal hydride inclusions. Contamination of the sample by moisture or by residual cleaning solvents can easily become a serious source of error when dealing with such small amounts of hydrogen. Incomplete separation of carbon dioxide and water in trap T_1 has been suspected when 20 γ or more of hydrogen were involved. However, a succession of refreezing, evacuation, and reheating of the condensate failed to show any evidence that carbon dioxide cocondensed with the water during the analytical run.

Table III gives recovery data obtained on methane, hydrogen gas, and sucrose, using the horizontal combustion tube and the less sensitive manometer. The methane and the hydrogen gas were added via the dosing stopcock used for calibration; consequently the analyses were unaffected by a boat blank. The sucrose samples were added to quartz boats as aliquots of an aqueous solution, evaporated to dryness, and placed in the combustion tube for burning. Although hydrogen gas in larger amounts had been used to calibrate the apparatus, the hydrogen gas analyses reported in this table were made to test the recoveries in the submicrogram range.

In Table IV is given a comparison of the results obtained by this method with those of the Pregl macrogravimetric method for the analysis of a pulverized zirconium hydride. For the micromethod the samples were weighed into a tiny platinum boat using a microbalance located in a room of controlled temperature and humidity.

Table IV. Results on Zirconium Hydride

(Horizontal tube and less sensitive manometer used)

Macromethod, Gravimetric		Micromethod, Manometric	
Sample, grams	Hydrogen, %	Sample, mg.	Hydrogen, %
1.008	1.97	1.421	1.94
1.017	1.99	0.523	2.01
1.013	1.98	1.280	1.92
1.002	1.97	1.381	1.90
1.004	1.97	0.473	1.94
Av.	1.98	1.436	1.94
Std. dev.	±0.01	1.156	1.98
		0.556	1.94
		0.952	1.90
		Av.	1.94
		Std. dev.	±0.04

Representative results that have been obtained on uranium metal, using the sensitive swivel-jointed manometer and both types of combustion tubes, are shown in Table V. The precision of these results, as indicated by the standard deviation from the mean, should not be taken as a quality of the method alone, but also of the material analyzed, which was added as 1-gram cubes. The use of chips or turnings might have afforded more representative sampling; however, it was necessary to use single chunks in order that the surfaces might be properly cleaned prior to analysis.

Two methods of cleaning were employed. One was to remove all surfaces of a thoroughly cleaned sample with a degreased smooth file; and the other was to electropolish the sample anodically for 1 minute at 8 volts in a bath of 46% sulfuric acid and 6% glycerol. After the electropolished sample was thoroughly rinsed with distilled water and fresh C.P. acetone, it was dried for 1 minute in the hot air stream of the heat gun, while tumbled in a small platinum basket about 8 inches away from the gun. Each sample was placed in the apparatus for analysis within 15 minutes after cleaning was begun.

Analyses of some of the high-purity uranium samples (Table V) illustrates the usefulness of the high sensitivity afforded by the stability of the blank (Table II). An estimate of the accuracy of the method may be obtained from the earlier data shown in Tables III and IV.

APPLICABILITY OF METHOD

The method is applicable to a variety of materials which burn easily in oxygen at 1000° C. The sample is not subjected to low pressures as in vacuum fusion and other methods. Large samples may be analyzed for micro quantities of hydrogen, or micro samples for higher percentages. The quartz combustion tube may be either horizontal with versatile boat loading, or vertical with increased sensitivity. If oxides of nitrogen are released in the combustion of the sample, provision should be made for their removal between the combustion tube and the hydrogen manometer. A method for such removal has been described by Naughton and Frodyma (*9*).

Table V. Typical Results on Various Uranium Samples

(Swivel-jointed manometer used)

Horizontal Combustion Tube		Vertical Combustion Tube	
Uranium sample[a]	Hydrogen found, p.p.m.	Uranium sample[a]	Hydrogen found, p.p.m.
ANL (A)	2.2	ANL (C)	0.75
	1.8		1.09
	1.8		0.96
	1.9		0.97
	1.0		0.76
Av.	1.7		1.05
Std. dev.	±0.4		1.02
ANL (B)	1.2		0.80
	1.7		0.91
	1.7	Av.	0.92
	1.5	Std. dev.	±0.13
Av.	1.5	ANL Hi-Purity (D)	0.06
Std. dev.	±0.2		0.06
BMI, dehydrogenated	0.0		0.15
	0.3		0.06
	0.4	Av.	0.08
	0.4	Std. dev.	±0.04
	0.4	ANL Hi-Purity (E)	0.09
	0.3		0.07
Av.	0.3		0.07
Std. dev.	±0.2		0.06
BMI, hydrogenated	6.3	Av.	0.07
	6.1	Std. dev.	±0.01
	6.6	ANL Hi-Purity (F)	0.04
	6.7		0.06
	6.3	ANL Hi-Purity (G)	0.06
	6.5		0.12
	6.1	ANL Hi-Purity (H)	0.14
	5.8		0.14
Av.	6.3		
Std. dev.	±0.3		

[a] ANL, Argonne National Laboratory sample; BMI, prepared by Battelle Memorial Institute.

The manometer should be useful in measuring microgram quantities of water as moisture or as hydrate in samples being dehydrated in a dry gas stream.

The manometer might also be used for collection and sensitive measurement of carbon dioxide, using liquid nitrogen as the coolant. The manometer used in the work would give a reading of 8.5 mm. per microgram of carbon. It would be necessary to precede the cold trap of the manometer with a tube of anhydrous magnesium perchlorate and to throttle the gas flow at stopcock S_4.

ACKNOWLEDGMENT

The author wishes to express appreciation to Bernhard Blumenthal of the Metallurgy Division, Argonne National Laboratory, for making available most of the uranium samples examined during this work, and to M. W. Mallett of Battelle Memorial Institute, Columbus, Ohio, for two uranium samples specially prepared for hydrogen study.

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RECEIVED for review January 6, 1956. Accepted April 5, 1956. Division of Analytical Chemistry, 129th Meeting, ACS, Dallas, Tex., April 1956. Work performed under the auspices of the U. S. Atomic Energy Commission.

Simultaneous Microdetermination of Copper and Iron Using Mixed Phenanthrolines

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Microgram quantities of copper and iron may be determined simultaneously by analysis of the mixed phenanthrolines in either one or two phases. The components are determined in water or isopentyl alcohol by simultaneous mathematical equations, or the copper is extracted as the cuprous-neocuproine complex in isopentyl alcohol while the ferrous-1,10-phenanthroline complex remains in the aqueous phase. Fairly reasonable analytical accuracy is obtained when both substances are in the same phase, while good analytical accuracy is obtained in the two-phase system where the extracted substance has a highly favorable partition coefficient.

THE ability to determine micro amounts of both copper and iron simultaneously by relatively simple and yet reliably accurate techniques would find applications in several instances (*8, 18, 20*). In clinical chemistry, for example, the existence of a metabolic interrelationship between serum copper and iron (*2, 6, 7, 9*) and the importance of either in various pathological states (*11, 19*) have led to an ever-increasing need for this type of determination. The specificity of several of the phenanthrolines (*4, 13, 15, 16*) has made absorptiometric methods possible for the simultaneous determination of both cations.

There are many precedents for the use of simultaneous analysis. It may involve the determination of one compound in a mixture (*1*) or the individual determination of two or more components in a mixture (*3, 5, 10, 12, 14*); the latter type has included the determination of as many as six components in a mixture (*17*).

Because the cuprous-2,9-dimethyl-1,10-phenanthroline (neocuproine) and the ferrous-4,7-diphenyl-1,10-phenanthroline (bathophenanthroline) complexes can be extracted from aqueous solution, whereas the ferrous-1,10-phenanthroline complex cannot be extracted, a variety of techniques can be considered for simultaneous determination.

The following three procedures were investigated: (1) the determination of the ferrous-1,10-phenanthroline and cuprous-neocuproine complex mixture in the aqueous phase by simultaneous mathematical equations (*3, 12*); (2) a similar method for the ferrous-bathophenanthroline and cuprous-neocuproine complexes in an organic extract phase (*3, 12*); and (3) the individual measurement of the ferrous-1,10-phenanthroline complex in the water phase and the cuprous-neocuproine complex in the organic phase.

The three methods were investigated with solutions containing copper and iron in the concentration range from 0 to 5 p.p.m., such as might be encountered in normal and pathological serum, a medium which is being currently investigated in this laboratory.

REAGENTS

Buffer solution. Weigh 57 grams of anhydrous sodium acetate and dissolve in distilled water in a 1-liter volumetric flask. Add 17 ml. of glacial acetic acid from a pipet and dilute to almost 1 liter. Adjust the pH to 5.0, dilute to the mark, and mix well.

Buffered 1,10-phenanthroline. Dissolve 75 mg. of 1,10-phenanthroline in 100 ml. of the buffer solution with vigorous shaking. Refrigerate.

Buffered 2,9-dimethyl-1,10-phenanthroline (neocuproine). Dissolve 75 mg. of neocuproine in 100 ml. of the buffer solution with vigorous shaking. Refrigerate.

4,7-Diphenyl-1,10-phenanthroline (bathophenanthroline). Weigh 75 mg. of bathophenanthroline and dissolve in 100 ml. of isopentyl alcohol. Refrigerate when not in use. The solution is stable for long periods.

Stock copper standard. Weigh 100 mg. of pure copper metal and dissolve in acidified copper-free water, with heating if necessary; dilute to the mark in a 1-liter volumetric flask.

Stock iron standard. Weigh 100 mg. of pure iron wire, dissolve in acid solution with heating and dilute to the mark with iron-free distilled water in a 1-liter volumetric flask.

Calibration standards. Prepare a series of standards for both copper and iron by pipetting 0.0, 1.0, 2.0, 3.0, 4.0, and 5.0 ml. of the stock solution into 100-ml. volumetric flasks. Dilute to the mark with contaminant-free distilled water, and mix well.

Solid ascorbic acid.

Isopentyl alcohol.

PROCEDURE

Preparation of Calibration Curves. COPPER (NEOCUPROINE). Pipet 3.0 ml. of each of the copper calibration standards into clean, dry, glass-stoppered centrifuge tubes. Add 3.0 ml. of buffered neocuproine solution and a spatula tipful (about 50 mg.) of solid ascorbic acid. The amount is not critical and a liberal excess does not appear to interfere with either the reaction or the

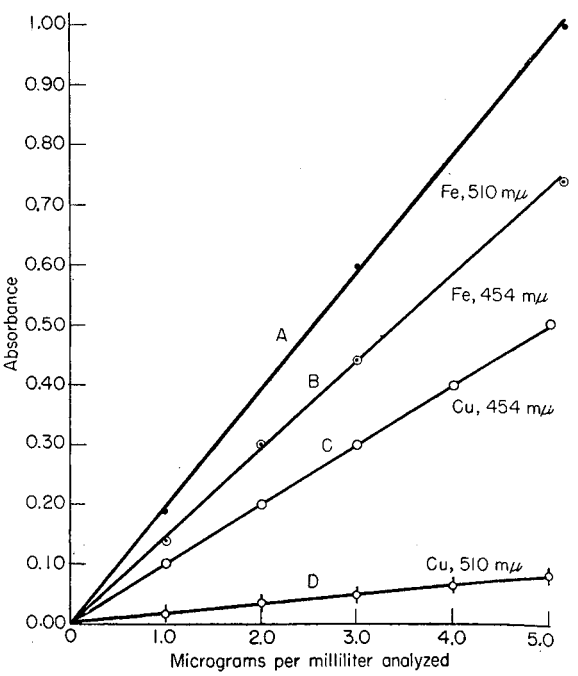

Figure 1. Calibration curves for cuprous-neocuproine and ferrous-1,10-phenanthroline complexes in aqueous solutions at wave length-absorbance maxima

extraction. If measurement in the aqueous phase is desired, compare the resulting solution to a blank at the proper wave lengths (454 and 510 or 535 mμ). If extraction is to be carried out, add 3.0 ml. of isopentyl alcohol to the solution, stopper the tube, and then shake it vigorously for 3 to 5 minutes. To separate the two immiscible phases, centrifuge the mixture at 3500 r.p.m. for 10 minutes. Carefully pipet off the isopentyl alcohol upper layer into 1-cm. Beckman cells.

Enough liquid need not be obtained to cover the window because 2 to 3 drops of water from the lower phase can buoy up the alcohol fraction if more volume is desired. The favorable partition coefficient obviates any loss back to the water phase, and enables one to use the water as a sort of jack in the presence of an inadequate volume of the alcohol phase. In practice, a little more than 2.0 ml. is pipetted off and 2 to 3 drops of copper-free water is then added along the frosted side of the cell. Measure the absorbances against the blank standard at 454 mμ and 510 or 535 mμ.

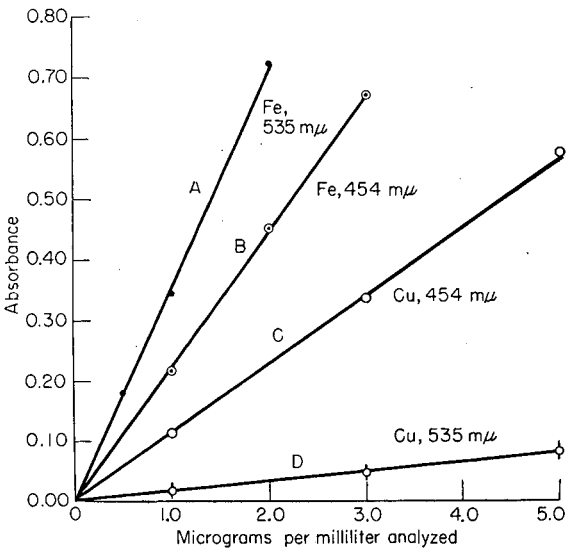

Figure 2. Calibration curves for cuprous neocuproine and ferrous-bathophenanthroline complexes in isopentyl alcohol solution at wave length–absorbance maxima

Iron (1,10-Phenanthroline). Pipet 3.0 ml. of each iron calibration standard into clean, dry, 1.9-cm. cells, then add 3.0 ml. of buffered 1,10-phenanthroline solution and a spatula tipful of ascorbic acid. Measure the absorbances of the well-mixed solutions at 510 and 454 mμ, using a test tube adapter on the Beckman DU.

Iron (Bathophenanthroline). Pipet 2.0 ml. of each of the iron calibration standards (up to the 3-p.p.m. standard) into clean, dry, glass-stoppered centrifuge tubes, and add 2.0 ml. of the buffer solution and a spatula tipful of solid ascorbic acid. Add 3.0 ml. of the isopentyl alcohol–bathophenanthroline solution, shake the stoppered tubes vigorously for 3 to 5 minutes, and centrifuge for clarification at 3500 r.p.m. for 10 minutes. Pipet off the red alcohol layer, observing the same considerations as described previously for the neocuproine method. Measure the absorbance of each solution at 454 and 535 mμ against the blank standard.

Analysis of Unknown Solutions. In order to analyze an unknown solution using either the first or third method, add 3.0 ml. of a 1 to 1 mixture of the buffered 1,10-phenanthroline and neocuproine solutions to 3 ml. of unknown. After reduction with ascorbic acid, simultaneous equations can be employed to determine both substances, or isopentyl alcohol can be used to extract the cuprous-neocuproine complex as described, thus allowing each substance to be determined quantitatively in separate phases (*3*, *12*).

In order to analyze by the second method, add 3 ml. of the buffered neocuproine solution and 3 ml. of the bathophenanthroline solution to 3 ml. of the unknown, which has been treated with ascorbic acid. The extraction and simultaneous measurement can then be carried out (*3*, *12*).

If desired, either one of the two cations can be measured separately in the same system by the use of the correct buffer alone or along with either isopentyl alcohol or bathophenanthroline in isopentyl alcohol.

DISCUSSION AND RESULTS

In order to obtain the working conditions for simultaneous mathematical analysis, it is necessary to calculate the absorptivity for the copper and iron standards at the wave lengths used for the different mixtures (*3*, *5*, *12*). For the first method—measurement of both complexes in water—Figure 1 shows the calibration curves graphed for the cations. *A* and *B* are the lines for iron at 510 and 454 mμ, and *C* and *D* are the lines for copper at 454 and 510 mμ, respectively. The ferrous–1,10-phenanthroline complex yields a greater absorbance at the copper-neocuproine complex peak than does the latter at this peak. The same consideration for the ferrous-bathophenanthroline complex against the cuprous-neocuproine complex is shown in Figure 2, where again the absorbances obtained for iron are much greater than those for copper, even at the peak values for copper. Curves *A* and *B* are the calibration curves of the ferrous-bathophenanthroline complex at 535 and 454 mμ in isopentyl alcohol, while curves *C* and *D* are those for the cuprous-neocuproine complex at 454 and 535 mμ, respectively, in isopentyl alcohol.

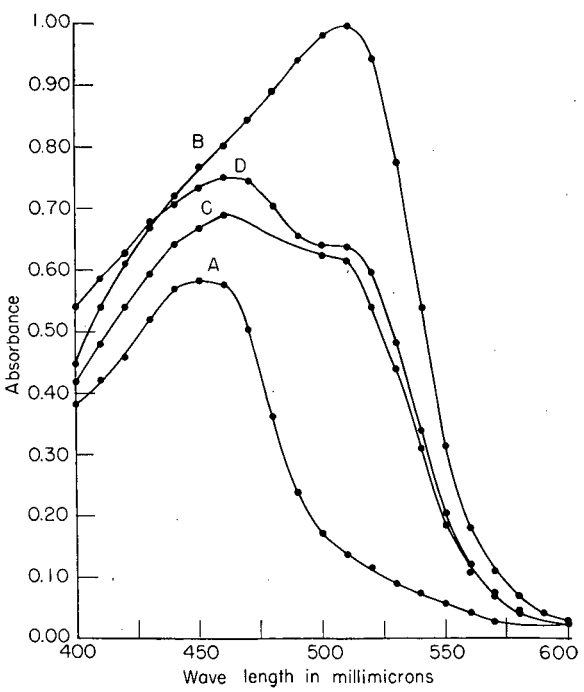

Figure 3. Absorbance–wave length plots in aqueous solution

A. Cuprous-neocuproine complex
B. Ferrous–1,10-phenanthroline complex
C. Artificial composite
D. Real mixture

Because mixtures of different complexing agents of similar chemical structure were to be used, it was decided to determine whether or not there would be any interference in color formation. The wave length–absorbance data for the curves of Figure 3 were therefore obtained. Curves *A* and *B* show the plots for curpous-neocuproine and ferrous–1,10-phenanthroline, respectively, in water.

The absorbance values of known concentrations of the iron and copper complexes obtained from individual spectral curves of each substance were added together for various wave lengths

to get the curve shown as *C*. When real mixtures of the two cations were then treated with equal amounts of both buffers to form complexes simultaneously in one medium and the wave length–absorbance plot was graphed, curve *D* was obtained. The concentrations of copper and iron employed in preparing curve *C* were slightly less than those used in preparing curve *D* so that the similar characteristics of the curves would stand out without being plotted coincidentally. The general shape of the curves, except in the small area where points were plotted for one curve and not the other, is similar within the limits of experimental error. More recently the similarity of the two curves has been confirmed with an automatic recording spectrophotometer. The close agreement in spectral characteristics for the artificial composite, curve *C*, and the real mixture, curve *D*, indicates that one does not interfere with the other when both complexes are formed together in the same solution.

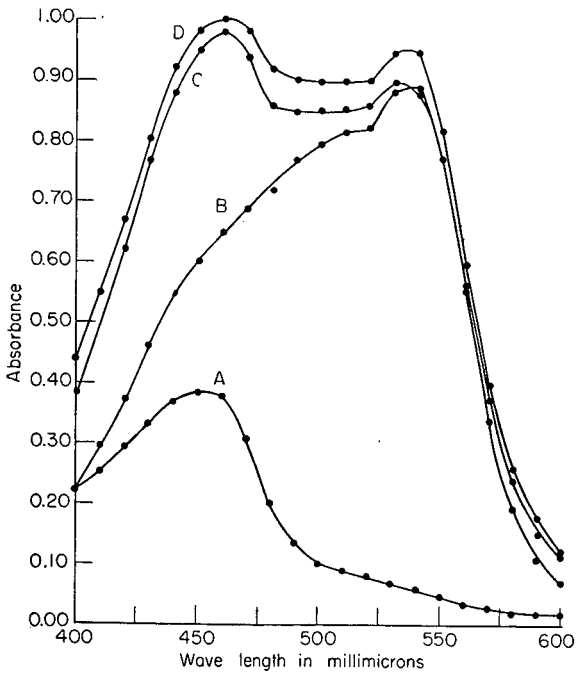

Figure 4. Absorbance–wave length plots in isopentyl alcohol

A. Cuprous-neocuproine complex
B. Ferrous-bathophenanthroline complex
C. Artificial composite
D. Real mixture

Table I. Recovery of Iron and Copper Using 1,10-Phenanthroline, Neocuproine, and Single Aqueous Phase

Copper, γ			Iron, γ		
Present	Found	Dev.	Present	Found	Dev.
2.0	2.0	0.0	10.0	10.4	+0.4
6.0	5.8	−0.2	10.0	10.8	+0.8
2.0	2.3	+0.3	8.0	8.6	+0.6
10.0	11.4	+1.4	2.0	2.1	+0.1
10.0	10.7	+0.7	6.0	6.3	+0.3
10.0	10.4	+0.4	10.0	10.1	+0.1
6.0	6.3	+0.3	2.0	1.9	−0.1
10.0	11.5	+1.5	2.0	1.9	−0.1
10.0	10.8	+0.8	4.0	4.5	+0.5
10.0	9.7	−0.3	6.0	6.5	+0.5
10.0	10.5	+0.5	8.0	8.0	0.0
10.0	10.0	0.0	10.0	10.0	0.0
2.0	2.0	0.0	10.0	10.5	+0.5
4.0	4.0	0.0	10.0	10.4	+0.4
6.0	5.5	−0.5	10.0	10.5	+0.5
8.0	7.7	−0.3	10.0	10.2	+0.2

A similar study was made on the ferrous-bathophenanthroline and cuprous-neocuproine complexes; the spectral data obtained for the isopentyl alcohol extracts are shown in Figure 4. Again curves *A* and *B* represent separate wave length–absorbance plots for the two complexes in the organic extract. Curve *C* represents the artificial composite, and curve *D*, a real mixture. The indication is that the formation of one does not interfere with the formation of the other when both complexes are formed at the same time and in the presence of excess complexing agent for both cations.

Table II. Recoveries of Iron and Copper Using Bathophenanthroline, Neocuproine, and Single Organic Phase

Copper, γ			Iron, γ		
Present	Found	Dev.	Present	Found	Dev.
5.0	4.7	−0.3	10.0	10.3	+0.3
5.0	5.0	0.0	6.0	6.0	0.0
5.0	4.4	−0.6	3.0	3.0	0.0
5.0	4.9	−0.1	2.0	2.0	0.0
10.0	10.7	+0.7	5.0	5.0	0.0
6.0	6.0	0.0	5.0	4.7	−0.3
3.0	2.7	−0.3	5.0	5.0	0.0
2.0	2.0	0.0	5.0	4.7	−0.3
4.0	4.3	+0.3	6.0	6.3	+0.3
6.0	5.7	−0.3	6.0	6.5	+0.5
10.0	9.5	−0.5	2.0	2.2	+0.2
10.0	10.3	+0.3	8.0	8.2	+0.2
2.0	2.1	+0.1	6.0	5.7	−0.3
6.0	5.7	−0.3	2.0	2.2	+0.2
10.0	9.6	−0.4	2.0	1.9	−0.1
10.0	9.7	−0.3	6.0	6.6	+0.6
10.0	9.7	−0.3	4.0	4.1	+0.1

Table III. Recovery of Iron and Copper Using 1,10-Phenanthroline, Neocuproine, and Mixed Aqueous-Organic Phase

Present, γ		Found, γ		
Cu	Fe	Cu	Fe	Dev.
7.5	0.0	7.5	...	0.0
7.5	5.0	7.6	...	+0.1
7.5	10.0	7.6	...	+0.1
7.5	15.0	7.7	...	+0.2
7.5	20.0	7.4	...	−0.1
0.0	7.5	...	7.4	−0.1
5.0	7.5	...	7.4	−0.1
10.0	7.5	...	7.6	+0.1
15.0	7.5	...	7.6	+0.1
20.0	7.5	...	7.4	−0.1
5.0	0.0	5.0	0.0	...
5.0	5.0	5.0	4.7	...
5.0	10.0	4.6	9.3	...
10.0	5.0	9.9	4.7	...
5.0	5.0	4.8	4.7	...
0.0	5.0	0.0	4.7	...

In Figures 3 and 4 the copper absorbance in the iron determination is very small at the iron peaks, while the iron absorbance in the copper determination is large. The advantages to be considered, however, in extracting both cations are those of concentration, purification or the separation from interfering substances, and the possibility of determining another cation or an anion in the aqueous phase without resorting to a new sample.

Recovery studies were carried out for the three procedures described. Tables I and II show the accuracy that can be obtained using simultaneous equations on mixtures of copper and iron. The recoveries appear to be analytically accurate for the ranges involved, although it is apparent that they are not so good as those shown in Table III, where the cations were measured individually after extracting them into separate phases. The disadvantage of measuring both substances in one phase is that the effective copper blank is increased by the much greater absorbance of the iron. However, the data obtained in Tables I and II, where copper was made both the major and the minor constituent, show that the recovery is analytically adequate.

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RECEIVED for review February 23, 1956. Accepted April 25, 1956. Supported in part by a Grant-in-Aid from the Receiving Hospital Research Corp.

Ter Meulen Micromethod for Direct Determination of Oxygen

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The ter Meulen method has been adapted to the direct determination of oxygen in organic compounds (sample size ranging from 3 to 10 mg.) and on the surfaces of various carbons containing 0.01 to 4.0% of oxygen (sample size ranging from 50 to 800 mg.). In a series of consecutive determinations, the time required for one determination is 15 minutes.

A NUMBER of papers published in recent years indicate that carbon-oxygen surface complexes are of interest to people working in a variety of research fields. In order to correlate a given property with the amount of carbon-oxygen surface complex, it is necessary to have a microanalytical method for the determination of the complex. Several methods have been proposed, but each one has some drawback.

Oxygen can be determined satisfactorily by difference only if the oxygen content is relatively high (*12*) and if the carbon, hydrogen, and ash content is known. With carbons of high sulfur or nitrogen content or containing relatively small amounts of oxygen, one must also know the sulfur and nitrogen content. The Unterzaucher method (*11*) has been used (*10*) for carbon blacks, but it is unsatisfactory because the oxygen is evolved in a reasonable length of time only from those blacks which have a relatively high hydrogen content, and the presence of ash introduces an error with furnace blacks. McDermot and Arnell (*2*) determined the oxygen content of charcoal surfaces by passing hydrogen over the samples (5 to 7 grams) heated to 1000° C. and determining water, carbon dioxide, and carbon monoxide in the effluent gases. Difficulty was encountered with the large amount of water produced and with incomplete conversion of the carbon monoxide to carbon dioxide in analysis.

This paper describes a ter Meulen micromethod (*4*) as it was developed for application to some carbons used in this laboratory. The method works equally well with pure organic compounds. In the ter Meulen method the sample is heated in a stream of hydrogen and any oxides of carbon which are produced are catalytically hydrogenated to methane and water; the water is a measure of the oxygen in the original sample. Russell and Fulton (*5*) greatly improved the original method by replacing the platinized asbestos with platinized quartz for a cracking surface and by replacing the nickelized asbestos with a highly active thoria-promoted nickel methanation catalyst. The main disadvantages of the Russell-Fulton modification are the time needed for each analysis (at least 1 hour), the care needed to vaporize the organic compound in the first half hour, and the frequency of catalyst regeneration (usually every 10 to 15 analyses, but as often as every four to five analyses for certain compounds which carbonize the platinized quartz). In many cases the sample size, 100 to 300 mg., may be excessively large.

In the present work the sample size has been reduced to 4 to 15 mg. of organic compound and the hydrogen has been specially purified to minimize the size of the blank. These changes reduce the analysis time to 15 minutes, reduce the care needed for sample vaporization, and make reactivation of the catalyst a very infrequent operation. In the case of the carbon samples, hydrogen removes only the carbon-oxygen surface complexes and practically all of the carbon remains at the end of the analysis. Organic compounds are generally completely vaporized.

EXPERIMENTAL

One black and two charcoals were used in this work.

Graphon. A partially graphitized carbon black (supplied through the courtesy of the Godfrey L. Cabot Co.) was made by heating Spheron Grade 6 (a medium processing channel black) to approximately 3000° in an electric furnace. The surface area is about 80 square meters per gram and its ash content is about 0.02%.

Su-60. A sugar charcoal of extremely low ash content was prepared, starting with confectioner's AA sugar furnished through the courtesy of the California and Hawaiian Sugar Refining Corp. This sugar was used because it had an ash content of 0.0008%. After activation (*9*) this charcoal had a BET area of 1020 square meters per gram, using ethyl chloride, and its ash content was less than 0.005%.

B. An activated commercial nut charcoal, de-ashed with hydrochloric acid in a Soxhlet extractor, dried and heated to 1000° in vacuo. The BET surface area is 1050 square meters per gram and its ash content is about 0.2%.

Analytical Apparatus and Procedure. The reduction apparatus for the carbon-oxygen complexes consists of a clear quartz tube with dimensions standard for combustion tubes used in ordinary carbon-hydrogen microdetermination. For carbon samples whose size must be increased because of the small amount of oxygen present, the first 18 cm. of the reduction tube is replaced with a piece of quartz of larger diameter. In this work a piece 20 mm. in outside diameter was used. This tube is filled, starting at the exit end, with about 3 cm. of silver wool, about 8 cm. of nickel-thoria catalyst (about 6 grams), and finally about 13 cm. of platinized quartz (20 mesh). The nickel-thoria catalyst and platinized quartz are prepared as described by Russell and Fulton (*5*). A standard absorption tube filled with Anhydrone is connected to the exit end of the reduction tube, and a flowmeter and guard tube are connected to the exit end of the absorption tube. The nickel-thoria section of the reduction tube is fitted with an electric furnace and adjusted for running at 350° (400°

for initial reduction or regeneration). The platinized quartz section is fitted with an electric furnace for running at 1000°.

If a source of special high purity hydrogen is available, this is connected to the side arm of the reduction tube, as in ordinary combustion analysis. In this work ordinary tank hydrogen was used and purified, as used, by passing it through a series of tubes arranged in the following order: Deoxo Purifier (Baker & Co. palladium catalyst); a 16-cm. electrically heated (350°) tube filled in the first half with platinized quartz and in the second half with nickel-thoria catalyst; a 16-cm. tube filled in thirds with Anhydrone, Ascarite, and then with Anhydrone again: and finally a 50-cm. tube filled in thirds with Anhydrone, Ascarite, and Anhydrone. The tubes used for this purpose were made from pieces of 11-mm. Corning 172 combustion tube. There appears to be no need for the nickel-thoria catalyst tube, but the tank hydrogen used in this work contained a trace of some hydrocarbon which, without prior removal, made the blanks much too high. The short Anhydrone-Ascarite tube needs relatively frequent replacement, and the long tube merely serves as an extra precaution. In order to create a nitrogen atmosphere around the mouth of the reduction tube, a 6.5-cm. funnel is placed beneath the mouth of the tube and a strong current of nitrogen is passed through it just before the sample is introduced to (or the boat removed from) the hydrogen stream. This procedure prevents "flashbacks," which otherwise occur rather easily.

All samples are introduced from a weighing piggy filled with nitrogen. To facilitate the transference and to prevent the influx of air, the weighing piggy and reduction tube are of the same diameter, with ends ground square. With the end of the piggy butted against the reduction tube, the boat is pushed or pulled from one to the other with a suitable glass rod. Proper operation of the reduction train is checked with pure benzoic acid (Parr calorific grade) before each day's runs.

The following preparative procedure was adopted for the oxygen determinations in the carbon samples, in order to remove physically adsorbed moisture and to prevent take-up of oxygen from the air.

The carbon samples, in porcelain boats, were outgassed at 110° in vacuo (with continuous pumping) for 12 hours, cooled to room temperature in vacuo, surrounded with nitrogen at 1 atm., transferred to weighing piggies in a stream of nitrogen gas, stoppered, and then weighed. After analysis, the boat was again weighed in the same piggy with nitrogen; the sample weight was obtained by difference. The organic compounds (contained in platinum boats) were weighed in weighing piggies thoroughly flushed out with nitrogen. All weighings were done in the presence of polonium to eliminate error due to static charges.

Even when not in use the nickel-thoria catalyst furnaces were kept at 350°, and a hydrogen flow of about 5 cc. per minute was maintained. About 15 minutes before making determinations, the platinized quartz section was raised to about 1000°, the Anhydrone absorption tube was put in place, and the hydrogen flow rate was adjusted to 75 cc. per minute. A sample, in a boat, was weighed in a weighing piggy in a nitrogen atmosphere (carbon samples were weighed as in the preceding paragraph). The absorption tube was removed, flushed with 250 cc. of dry air, wiped with a chamois, and weighed. It was weighed again in 5 minutes.

It was found helpful to follow a time schedule for each operation. This schedule ran as follows for a blank and a sample.

1:00. Attach weighed absorption tube and commence heating sample chamber.
1:13. Discontinue heating of sample chamber.
1:15. Remove absorption tube and connect flowmeter and guard tube directly to the reduction tube.
1:16. Flush absorption tube with 250 cc. of dry air at 50 cc. per minute, using aspirator.
1:17. Cool sample chamber to room temperature with compressed air and add sample via piggy, using nitrogen atmosphere.
1:21. Remove absorption tube from aspirator and wipe with chamois.
1:22. Place absorption tube on balance pan.
1:23. Pour water back into aspirator and increase hydrogen flow rate to 150 cc. per minute to flush out any air that may have entered with the sample.
1:27. Weigh absorption tube (five swings; ignore the first two).
1:29. Remove absorption tube from balance; decrease hydrogen flow rate to 75 cc. per minute.
1:30. Attach absorption tube and commence heating sample in reduction chamber. Heat gently at first until sample is gone, then finally with the full blast of an air-gas torch. With carbon samples, vigorous heating may be used from the start. The degree of heating is gaged by the rate of formation of water—i.e., water should not be permitted to condense in capillary of absorption tube.
1:31 to 2:00. Follow exactly the same procedure, but at 1:47 remove the boat and add another sample if desired. A nitrogen atmosphere must be used.

A few suggestions concerning the use of this procedure may be helpful. A sample of benzoic acid should be used occasionally as a check on the performance of the catalyst in the reduction train. In order to run several samples in succession, it is simplest to weigh all the samples in advance, leaving each in its respective piggy. When the oxygen content is very low, it is advisable to alternate each analysis with a blank run, as was done in this work. A blank of 0.05 to 0.1 mg. of water for a 15-minute period at a flow rate of 75 cc. per minute is normally obtained. The blanks usually remain constant for a day, and sometimes for as long as a week. The precision of the blank determination is within ±0.01 mg.

If the blank becomes too high or if the check analysis on benzoic acid is in error, it is probable that the quartz has become carbonized or that the catalyst needs regeneration.

To remedy this, cool the reaction tube, flush out the hydrogen with air, then heat the quartz catalyst until the carbon has disappeared. Cool again, flush out the air with hydrogen, then reduce the catalyst at 400° overnight, using a hydrogen flow rate of about 50 cc. per minute. The blank should be about 0.05 mg. for a 15-minute interval at a flow rate of 75 cc. per minute.

RESULTS

The results obtained with three organic compounds are given in Table I and with a few typical carbon samples in Table II. Cholesterol was chosen because of its low oxygen content, 2-naphthol was chosen because of the sublimation difficulties mentioned by Russell and Fulton, and benzoic acid was normally used as a check because of its ready availability in highly purified form (Parr calorific grade). Only six of the benzoic acid samples shown in Table I weighed over 6 mg. The method itself has already been shown to be applicable to a wide variety of compounds. Additional results obtained with carbon-oxygen surface complexes are discussed elsewhere (*8*). Each carbon sample was outgassed at 110° for 12 hours prior to analysis, except where noted.

Table I. Oxygen in Organic Compounds

Compound	No. of Samples	Wt. of Sample, Mg.	Oxygen, % Exptl.	Oxygen, % Calcd.
2-Naphthol	1	9.98	11.2	11.1
Cholesterol	1	12.38	4.15	4.15
	1	12.93	4.25	4.15
Benzoic acid	2	3.06–4.04	25.8	26.2
	1	3.54	25.9	26.2
	7	2.53–9.62	26.0	26.2
	4	3.71–8.09	26.1	26.2
	3	3.91–7.77	26.2	26.2
	3	2.53–4.03	26.3	26.2
	6	2.82–7.08	26.4	26.2
	3	3.65–5.16	26.5	26.2

DISCUSSION

The ter Meulen micromethod should be applicable to compounds containing sulfur and, with small modification, to compounds containing nitrogen. Russell and Marks (*6*) have shown that the same catalyst (and in the same amount as used here), though poisoned by sulfur, will hold 200 to 400 mg. of sulfur before it fails to give quantitative conversion of oxygen to water. This would correspond to an enormous number of analyses using 10-mg. samples. Once the catalyst is sulfur-poisoned it is not practical to regenerate it; a new charge of catalyst is used instead. With nitrogen-containing compounds Russell and

Table II. Oxygen in Carbon Samples

Sample	Wt. of Sample, Mg.		Oxygen, %
Graphon, as supplied	504.3		0.0176
	826.4		0.0129
		Av.	0.0153
Graphon, outgassed	623.2		0.0043
	553.9		0.0032
		Av.	0.0038
Graphon, poured through air at 700°	390.4		0.312
	260.1		0.342
		Av.	0.327
Su-60, as made	189.8		0.412
	195.1		0.428
		Av.	0.420
Su-60, outgassed	221.8		0.368
	236.4		0.402
		Av.	0.385
B, as supplied	60.7		4.46
	50.6		4.23
		Av.	4.34
B, outgassed	114.4		4.41
	164.1		4.38
		Av.	4.39

Marks (*7*) found that nitrogen is evolved as nitrogen gas, ammonia, or both, and by replacing the Anhydrone tube with sodium hydroxide pellets they were able to retain quantitatively all the water produced without error due to ammonia.

To check the usefulness of Ascarite alone, 13-mg. samples of dry phthaldiamide were gently heated in an unpacked combustion tube with precautions taken to prevent phthalimide from subliming into the absorption tube; the liberated ammonia was carried by dry nitrogen through a standard absorption tube filled with Ascarite. No increase in weight was observed. This shows that a standard absorption tube filled with Ascarite alone would be as satisfactory as Anhydrone for the determination of water, but its capacity would probably be less. It is believed that the results shown in Table II are not in error due to the nitrogen and sulfur content of the carbons, even though Anhydrone was used as a water absorbent. Any sulfur would have been retained by the nickel-thoria catalyst and the nitrogen content is presumably negligible. Studebaker (*10*), in making analyses on 39 different samples of carbon blacks, made the same assumption concerning nitrogen. Anderson and Emmett (*1*) have shown that the small quantity of nitrogen complexes formed by treating charcoal with ammonia at 750° to 900° are more stable than oxygen complexes. In a study of the thermal decomposition products they showed that most of the oxygen complex decomposed in the range 600° to 800°, whereas the nitrogen complex decomposed in the range 900° to 1200° and constituted about 1% of the total gases removed. Nitrogen complexes are not formed by reaction of molecular nitrogen with charcoal.

It is unlikely also that there is error caused by ash, since the ash contents are 0.02% for Graphon, less than 0.005% for Su-60, and 0.2% for B, and only part of this ash in each case would be in the surface. In general it is unlikely that the siliceous part of the ash (the major part removed by hydrofluoric acid de-ashing treatments) would cause an error, for at the temperature used in the analysis neither carbon nor hydrogen will appreciably reduce silica.

The effect of metallic impurities in blacks cannot be easily assessed. Metallic oxides such as iron (common in channel blacks) in the surface would certainly be reduced by hydrogen or would react with carbon at the temperature of treatment in the ter Meulen tube. If prior to the analysis the carbon sample is raised to a temperature of 600° to 950°, such metal oxides would be reduced to a lower oxide, the metal, or the carbide by carbon itself (*3*) (depending on the temperature), and the effect of metal impurity would be either greatly reduced or eliminated. Alkaline earth or alkali metal impurities (common in furnace blacks) would probably be present as carbonates and the first of these would probably yield carbon dioxide at the temperature of treatment in the ter Meulen tube. However, their oxides would not be reduced by hydrogen nor would they react with carbon to form carbides at this temperature. In the case of charcoals it is unlikely that the ash causes any appreciable error in the results, for at the temperature at which charcoals are produced metallic impurities should be converted to noninterfering forms.

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RECEIVED for review December 12, 1955. Accepted April 25, 1956. Progress report of work done under Contract N8onr54700 with the Office of Naval Research.

Bioassay for the Estimation of Metal Ions

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The method involves preparation of progressively diluted aliquots of an unknown solution containing the cation to be estimated. The toxicity of these aliquots for the test organism is compared with the toxicity of standard solutions containing the same cation. An aliquot having the same toxicity as a particular standard solution is obtained. As solutions of equal toxicity must contain the same cation concentration, it is possible to calculate the unknown concentration from the observed dilution factor and the standard concentration.

ALTHOUGH bioassay techniques (*3*, *15*) have been of inestimable value in vitamin, nutritional, and other biochemical research, they have not been extensively applied to the solution of many traditionally chemical problems. Such methods can at times offer unique advantages; and, as Bacharach (*1*) and Harris (*6*) have pointed out, the tools and techniques of the bioassayist can occasionally be extremely valuable to the analytical chemist. In some instances, the sensitivity of these methods is approached only by the most refined physical measurements. Lowry and Bessey (*10*), for example, were able to determine 0.5 to 2.0 mγ (10^{-9} gram) of riboflavin with an over-all precision of 3 to 5% by a microbiological method. The literature contains numerous other equally impressive examples (*19*).

Table I. Cation Toxicities Using the Guppy as a Test Organism

Toxic Cation	Salt Used	LD_{50}, Mole/Liter
Ag^+	$AgNO_3$	4×10^{-8}
Hg^{++}	$HgCl_2$	1×10^{-7}
Cu^{++}	$CuSO_4$	3×10^{-7}
Cd^{++}	$Cd(NO_3)_2$	5×10^{-7}
Pb^{++}	$Pb(NO_3)_2$	1×10^{-6}
Zn^{++}	$ZnSO_4.7H_2O$	2×10^{-6}
Ni^{++}	$NiCl_2.6H_2O$	2×10^{-5}

Many metal ions are toxic to aquatic organisms (*4*, *8*, *17*). Experiments conducted in this laboratory (*9*, *18*) have indicated that the guppy (*Lebistes reticulatus*), a common aquarium fish, is killed by several different cations present at very low levels (Table I). The silver ion, for example, is highly toxic to this organism. If guppies are immersed for a 24-hour period in solutions approximately $4 \times 10^{-8}M$ or greater in silver ion, death results. Because few conventional analytical techniques are capable of detecting metal ions at this low level, an investigation of the applicability of this and similar findings to the estimation of silver ion and other toxic cations seemed to be of interest. It was also hoped that the usefulness of bioassay techniques in the approximate measurement of physicochemical quantities might be demonstrated. Both aims could be achieved by a determination of the solubility and solubility product constant of some slightly soluble salt containing a toxic cation. Such a measurement would be particularly appropriate if precise data obtained by other methods were available in the literature for comparison.

The basic principle involved in such a determination is simple. To find the solubility of a salt containing a toxic cation and a relatively nontoxic anion, it is only necessary to start with a saturated solution of the salt and dilute until a solution is obtained that kills the same fraction of guppies as is killed by a standard solution of the toxic cation under identical experimental conditions. The concentration of the toxic cation in the standard is then equal to the concentration of the toxic cation in the diluted sample of the saturated solution. Since the dilution factor is known, the concentration of the undiluted saturated solution can be calculated. This concentration will be the solubility. The results described below illustrate the application of this principle to a determination of the solubility of silver chloride in water, using the guppy as a test organism.

REAGENTS AND MATERIALS

Experimental Animals. Guppies (*Lebistes reticulatus*) of roughly the same age obtained from a local wholesale tropical fish concern were employed in the work. Since, under the experimental conditions used, results indicated that there was little if any sex difference in response to the various poisons, both males and females were used.

Water. Distilled water passed through a large capacity Dowex 50 exchanger was used in the bioassay procedure. Singly distilled water that had been passed through a similar exchanger was then triply distilled for the preparation of the saturated silver chloride solution.

Silver Chloride. Precipitation was effected by the dropwise addition of 0.1*N* C.P. silver nitrate to an equivalent amount of C.P. sodium chloride. The precipitate was hot digested for 24 hours. During the following 2 days the precipitate was repeatedly washed by decantation. The fourth day it was filtered and washed, and then suspended in triply distilled water. The suspension was placed in a thermostat maintained at 25.0° ± 0.05° C. and continuously stirred for 24 hours. The saturated silver

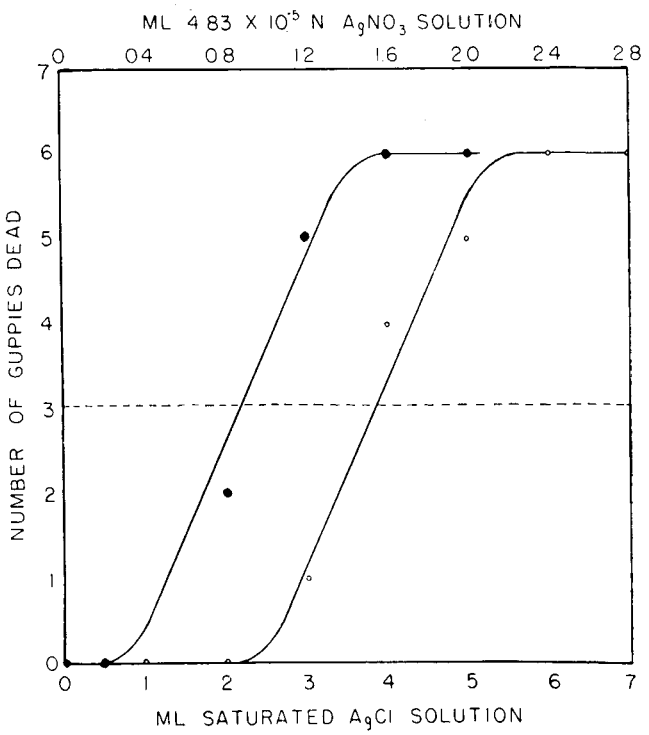

Figure 1. Toxicity as a function of the volume of added metal ion

● **Standard silver nitrate**
○ **Saturated silver chloride**
Data illustrate determination of solubility of silver chloride by bioassay

chloride solution was then drawn off through a sintered-glass filter and stored in an opaque bottle. All operations were carried out in the dark or in subdued light.

All other reagents were of analytical reagent grade or conformed to ACS specifications.

PROCEDURE

The concentration of the various metal ions necessary to kill one half of the test organisms (LD_{50}) under the given experimental conditions was determined as follows:

Standard solutions differing by steps of a factor of ten were first prepared—e.g., silver nitrate $10^{-7}M$, $10^{-8}M$, $10^{-9}M$, etc.

Three guppies were subjected to 2 liters of each of these solutions for a 24-hour period. Controls containing no added ions were always run simultaneously. In general, all the guppies died in solutions more concentrated than a particular value—e.g., $10^{-8}M$—while those in less concentrated solutions—e.g., $10^{-9}M$ or less—and those in the blank survived.

Standard solutions differing by a single unit or several units were next prepared—e.g., $2 \times 10^{-8}M$, $4 \times 10^{-8}M$, etc.—and treated as in the step above. By this means it was possible to obtain again an upper and lower limit for the desired value.

By a repetition of the process described above the approximate LD_{50}'s for the various cations were obtained and recorded in Table I. About 1000 guppies were used to obtain the data in this table.

In the solubility determination the approximate toxicity of the solutions employed was first established by experiments similar to those described above. Solutions were prepared containing 2, 3, 4, and 5 ml. of saturated silver chloride per liter of water. Four liters of each of these solutions were then placed in beakers. Standard solutions containing 0.4, 0.8, 1.2, and 1.6 ml. of $4.83 \times 10^{-5}M$ silver nitrate per liter were also prepared. Four liters of each of these were likewise placed in beakers. Six guppies were then added to each of the eight beakers. After 24 hours at room temperature (23° ± 3° C.) the number of dead guppies in each beaker was recorded and plotted against the milliliters of reagent used. From Figure 1 the number of the milliliters necessary to kill one half of the guppies was determined. From the milliliters of both solutions needed to kill one half of the guppies and from the molarity of the silver nitrate solution, the molarity of the undiluted saturated silver chloride was calculated. The entire process was repeated ten times and the collected results are shown in Table II.

The procedure outlined is not limited to solubility determinations or to estimations involving the silver ion. The data in Table I can be used to approximate the range required for standard solutions to be used in the estimation of other cations by procedures similar to that employed for silver.

The pH of all the solutions to which the fish were exposed lay between 6.1 and 6.4. The fish were not fed or aerated during the 24-hour experimental period, and the beakers containing the fish in the experimental solutions were loosely covered with a watch glass to prevent accidental contamination by dust, etc. Special care was taken to prevent contamination of the glassware by concentrated toxic metal solutions. Use of new beakers and leaching in distilled ion exchange water gave satisfactory results.

RESULTS

The approximate LD_{50}'s for the various cations are recorded in Table I. As the bioassay technique described is based on the measurement of a dilution factor needed to give a solution of the same toxicity as a standard solution, it is clear that the data in Table I represent the lowest concentrations of the respective cations that can be detected by this method. It is also obvious that the technique cannot be directly applied to: solutions containing both toxic anions—e.g., CN^-—and toxic cations, complex mixtures of toxic cations and solutions containing other substances that are toxic to guppies.

Figure 1 depicts a typical set of curves obtained in the solubility determination. From the abscissas corresponding to the points formed by the intersection of the dotted line with the two curves, the solubility can be calculated. The similarity of these curves to conventional titration curves is noteworthy. The procedure can, in fact, be likened to a titration with standard silver nitrate using the experimental animal to indicate the end point. The results of several such "titrations" are displayed in Table II.

Table II. Solubility of Silver Chloride in Water

Detn. No.	$AgNO_3$, Ml.[a]	AgCl, Ml.[b]	Solubility, Moles/Liter $\times 10^{+5}$
1	0.69	2.5	1.3
2	0.86	4.2	1.0
3	0.74	3.9	0.9
4	0.84	3.9	1.0
5	0.69	3.0	1.1
6	0.84	4.1	1.0
7	0.90	4.3	1.0
8	1.20	3.75	1.5
9	1.40	4.25	1.6
10	0.83	3.5	1.1
Av.			1.2 ± 0.2[c]

[a] Milliliters of standard $4.83 \times 10^{-5}M$ $AgNO_3$ solution necessary to kill one half the guppies.
[b] Milliliters of AgCl solution necessary to kill one half the guppies.
[c] Average deviation.

DISCUSSION

The solubility of silver chloride found by the method employed in this work is $1.2 \pm 0.2 \times 10^{-5}$ mole per liter. Gladhill and Malan (*5*) determined the solubility conductometrically to be $1.334 \pm 0.005 \times 10^{-5}M$. This value agrees closely with the value obtained potentiometrically by Owens (*13*). Pinkus and Hanrez (*14*) have tabulated values obtained by various investigators which ranged from 1.14 to $1.53 \times 10^{-5}M$. Their own determination set the solubility at $1.58 \times 10^{-5}M$ in $0.1M$ potassium nitrate. Within its relatively large experimental error, therefore, the value obtained from this work compares favorably with the best values obtained by precise physical methods. The method possesses several important advantages: It requires no expensive apparatus and no elaborate techniques and as it involves the simultaneous direct comparison of a standard with an unknown, it is relatively independent of the biological variability of the test organisms.

The literature contains few examples of the application of bioassay techniques to the determination of physicochemical constants. One classical example of such methods is found in the work of Hastings and others (*7*, *11*), who determined the stability constant for the calcium citrate chelate by utilizing the effect of free calcium ion on the contraction of the ventricle of the isolated frog heart. If metal ion bound up in a particular chelate is not toxic to the test organism, the method described in this work could also be applied to the estimation of chelate stability constants.

Other methods for the bioestimation of metals are based on the fact that many of these are nutritionally essential trace elements. Consequently, if a medium is supplied with all essential nutrients except the particular metal under investigation, this metal becomes the growth-limiting factor. A calibration curve relating some function of extent of growth to metal ion concentration can then be constructed and unknown samples assayed. Many times this method is fairly specific for the metal involved. Snell has (*2*) employed bacteria for the quantitative determination of manganese in this fashion. Nicholas (*12*), using fungi, has reported important analytical work with several metals.

Various enzymes are strongly inhibited by metal ions (*16*, *17*), while others require metal ions for activation. Both of these facts could find significant application in the development of new analytical methods.

ACKNOWLEDGMENT

The authors gratefully acknowledge the generous grant from the University of Texas Research Institute that made this study

possible. They would also like to express their appreciation to Bernard Grushkin for permission to use some of his unpublished data in Table I.

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RECEIVED for review October 7, 1955. Accepted January 10, 1956.

Application of Karl Fischer Water Method to Oxidants, Reductants, and Amines

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Studies with the two-solution modification of the Karl Fischer method show that it is applicable in the same cases as the original method and has special advantages with reducing and oxidizing substances and amines. In contrast to the original Karl Fischer reagent, the two solutions are rather stable. Substitutes for pyridine and methanol were tried, but none was found to give better results.

SOME years ago a modification of the Karl Fischer method for the determination of water was published (*2*). Instead of using a single solution containing pyridine, methanol, sulfur dioxide, and iodine, the use of two solutions was recommended, one containing pyridine, methanol, and sulfur dioxide (Solution I) and the other containing iodine and methanol (Solution II). For titration according to this modification the sample is dissolved or suspended in the first solution, which is then titrated with the second. This paper deals with experience obtained in this laboratory using the method.

KEEPING QUALITIES OF SOLUTIONS

In the normal Karl Fischer solution there is a rapid fall in the water equivalent, partly caused by absorption of atmospheric moisture by the hygroscopic reagents and partly caused by side reactions. In the two-solution modification, Solution I undergoes a slow change even when kept in stoppered bottles. Usually its water content decreases (*2*, *4*); in one case the following water contents were determined after 0, 1, 2, 3, and 5 months: 0.027, 0.032, 0.019, 0.017, and 0.015%. This small change is, however, of no importance as a correction is automatically made in each titration.

Of greater significance is the permanence of Solution II. As Seaman, McComas and Allen (*4*) have shown, the standardization changes only because of absorption of moisture. It is therefore necessary to use effective drying tubes on the titration flask. Eberius (*1*) claimed, however, that the permanence of the solutions was not improved with the two-solution modification. This matter was therefore reinvestigated; in Figure 1 the changes in two samples, A and B, of Solution II are compared with the change in a normal Karl Fischer solution of the same strength [figures are taken from Eberius (*1*)]. Sample A was a solution used every day in routine work and was stored in a titration apparatus with large drying tubes (25 × 3 cm.). No change in water equivalence was observed during the first 14 days. After that period, most of the solution was used and only a small volume left. In this case small amounts of absorbed water have a greater effect on the strength and the water equivalence is decreased. Sample B was of the same origin as A, but used only for the standardizations. After 0, 1, and 2 months the respective *W*-factors were 1.727, 1.732, and 1.705. Thus, by effective protection from atmospheric moisture Solution II is nearly as constant as the standard solutions used in alkalimetry.

The iodine content of Solution II, determined by thiosulfate titration, changed very little with time. Sample A changed from 0.2368*N* on the fourth day to 0.2367 and 0.2388*N* after 14 and 30 days, respectively; sample B changed from the same value on the fourth day to 0.2368, 0.2371, and 0.2374*N* after 14 and 19 days and 2 months, respectively. This small increase in normality shows that the methanol slowly evaporates and that the effect was greatest for sample A when there was only a small volume left.

Use of a stronger Solution II may be more convenient—e.g., 60 to 70 grams of iodine per liter—in order to limit the volumes when the sample contains large quantities of water. The per-

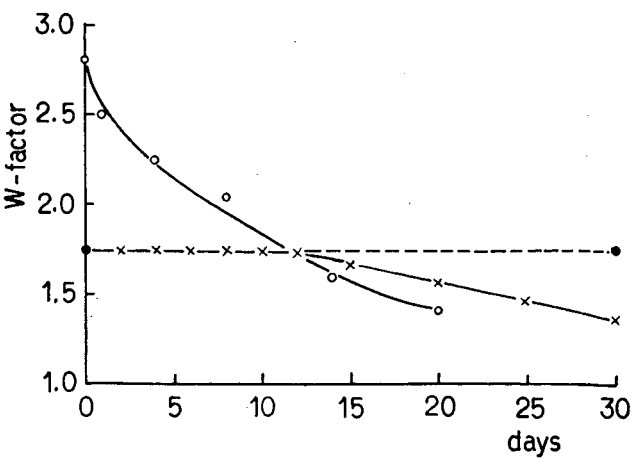

Figure 1. Comparison between stability of normal and modified Karl Fischer reagent

○. Normal
×. Sample A
●. Sample B

Table I. Determination of Water in Iodine-Consuming Substances

Compound	Compd. Added, Mg.	Water Added, Mg.	Solution II, Ml.	Ml. of Solution II for Oxidation	Water Found, Mg.	Water Calcd., Mg.
Method 1[a]						
Sodium thiosulfate pentahydrate	105.8	..	24.61	1.70	37.9	38.4
	106.2	..	24.76	1.73	38.1	38.5
	127.8	..	29.52	2.49	45.9	46.4
Potassium ethyl xanthate	97.6	40.5	26.38	2.55	40.5	40.5
	104.9	59.9	38.40	2.98	60.8	59.9
Ascorbic acid	111.5	32.0	22.08	5.08	32.9	32.0
Method 2[b]						
Sodium thiosulfate pentahydrate	95.8	..	21.90	1.64	34.6	34.8
	94.5	..	21.63	1.62	34.2	34.3
Ascorbic acid	166.4	37.8	32.30	8.04	38.1	37.8

[a] Weighed amount of sample (column 1) and water (column 2) was dissolved in 10 ml. of methanol-pyridine solution (1 to 1) and titrated with Solution II (N = 0.2347, W = 1.706, and water content 0.409 mg. per ml.) (column 4). Then 5 ml. of Solution I was added and the titration continued with Solution II (column 3).
[b] Weighed amount of sample (column 1) and water (column 2) was dissolved in 5 ml. of Solution I and titrated with Solution II (column 3). This value should be corrected for iodine consumption of sample (column 4).

centage decrease in the strength of such a solution from absorption of atmospheric moisture is minimized, but there is also a slight evaporation of iodine. As a net result the W-factor decreases about 2% during a month.

REDUCING SUBSTANCES

The original Karl Fischer method sometimes fails when the sample consumes iodine because the reaction between iodine and the iodine-consuming component is not quite stoichiometric and varies with titration conditions. When no precautions are used, the two-solution method suffers from the same limitations. Potassium xanthate gives, for example, varying results when the proportion of water to xanthate changes. In water-free conditions the iodine consumption is stoichiometric, but when the water content increases to over 30% the xanthate does not consume any iodine at all. In these cases a modified procedure is useful. In Table I some results with iodine-consuming substances are listed.

Procedure. The sample is dissolved in about 10 ml. of a mixture of equal parts of methanol and pyridine and then titrated with the iodine-methanol solution (Solution II) until the color changes to yellow. Then a measured quantity of Solution I is added, and the titration is continued until the color changes to brown. The value obtained is corrected for the water content of the solvents of Solution I and of Solution II. In this case it is convenient to use the normality instead of the W-factor of Solution II and to standardize it against standard sodium thiosulfate solution (a known amount of Solution II is added to an aqueous potassium iodide solution and titrated). The water content of Solution II is calculated from the difference between the stoichiometric water equivalence (from the thiosulfate titration) and the real water equivalence (from a standardization with water).

When the reaction between iodine and the iodine-consuming component of the sample is stoichiometric a shorter method may be used: An aliquot part of the sample is dissolved in methanol-pyridine solution and titrated with Solution II. The water content is then determined on another part of the sample, and this titration is corrected for the iodine consumption of the sample obtained from the first titration. The value is also corrected for the water content of that part of Solution II which is used for the oxidation.

OXIDIZING SUBSTANCES

It is also more convenient, when oxidizing substances are present, to use the normality of Solution II for the calculation, rather than the W-factor, and then to correct for the water content of the solvents (including that of Solution II) and for the iodine formed in the reaction between iodide ion and the oxidizing agent. However, many substances such as hydrogen peroxide require an acid medium for the oxidation

$$H_2O_2 + 2H^+ + 2I^- = I_2 + 2H_2O$$

In pyridine solution this equilibrium is thus displaced to left and the main reaction is oxidation of sulfur dioxide to sulfuric acid (*6*). Therefore, in these cases the water can be titrated without interference from the oxidizing agent.

Copper (II) and iron (III) salts are examples of substances which require correction.

AMINES

In the original Karl Fischer method, amines stronger than benzylamine ($K = 2.4 \times 10^{-5}$) must be neutralized by dissolving in acetic acid (*3*). With the modified reagent no such neutralization is necessary if the proportion of Solution I to amine is sufficiently large. As a rule, 10 ml. of Solution I can take about 0.5 gram of amine without interference. For example, a sample of 98.2% ethanolamine (determined by titration with standard hydrochloric acid) was found to contain 1.79 ± 0.02% water; when 43.7 mg. of water was added, 43.3 mg. were recovered. Other amines such as methylamine, ethylamine, dimethylamine, diethylamine, and ethylenediamine gave similar results.

When the water content is low it is necessary to weigh a greater amount of amine, and it is then advisable to add acetic acid, as otherwise the end point is not stable. The most convenient procedure is to add 10 ml. of acetic acid to 20 ml. of Solution II, titrate to the first end point, add the sample, and titrate again to the final end point. In this way an automatic correction for the water content in the acetic acid is obtained. With this procedure the following values were obtained with about 1.5, 1.5, 2.0, 3.0, 5.0, and 7.0 grams of the 98.2% ethanolamine: 1.80, 1.79, 1.78, 1.76, 1.74, and 1.76% water. Other amines tried included ethylenediamine, aniline, diphenylamine, and dimethylaniline. The end point with aniline is not entirely stable, and the brown color fades in about 1 minute, but this is not a serious limitation.

SUBSTITUTES FOR PYRIDINE AND METHANOL

The reasons for searching for substitutes for pyridine are its comparatively high price and the difficulty in observing the end point change from yellow to brown. Smith, Bryant, and Mitchell (*5*) have tried a variety of organic bases for the normal Karl Fischer reagent, but none was found to give stable solutions. As side reactions, which may be considered to be the cause of the poor stability, are of less importance in the two-solution modification, some experiments were made to find a better substitute for pyridine. Aniline and ethanolamine were tried but did not give stable end points. The only base tried which gave reproducible values was hexamethylenetetramine. The reaction here is the same as in the original Karl Fischer titration, except that the hexamethylenetetrammonium iodide is only slightly soluble and is precipitated (it is, however, not sufficiently insoluble to permit an acidimetric determination of water by filtration and titration with standard alkali).

The determinations were performed in the following way: Oven-dried hexamethylenetetramine (5 grams) was dissolved in 25 ml. of methanol, and the remaining water was titrated with a solution of 30 grams of iodine and 10 grams of sulfur dioxide per liter in methanol. Then a weighed amount of water was added, and the titration continued. The end point was sharp and stable, and the color of the solution changed from colorless to iodine yellow. Three such titrations gave W-factors of 1.789, 1.786,

and 1.791, for another solution W-factors of 1.702, 1.695, and 1.690 were obtained. The solution of sulfur dioxide and iodine was not stable, however, and after 14 days it was only pale yellow with a precipitate of sulfur on the bottom of the bottle.

Titrations where solutions of sulfur dioxide and hexamethylenetetramine were placed in the titration flask and titrated with an iodine-methanol solution did not give good results. The solutions turned yellow before the end point was reached when sulfur dioxide was in excess, and the results were very variable when hexamethylenetetramine was in excess.

As substitutes for methanol, other alcohols or glycols may be used in the two-solution modification as in the original method. None gave better results than methanol. With higher alcohols the pyridinium iodide is rather insoluble, which gives a possibility of acidimetric determination of water. In this case a small excess of an iodine-octanol solution was added to a weighed amount of water in a sulfur dioxide–pyridine-methanol solution, and the precipitate formed was filtered through a funnel with a sintered disk and washed with carbon tetrachloride. The precipitate was then dissolved in water and some ethanol to give a clear solution and then titrated with a standard sodium hydroxide solution. In a typical experiment the precipitate corresponding to 34.0 mg. of water consumed 49.30 ml. of 0.1105N sodium hydroxide solution, which gave 49.1 mg. of water. After correction for water in the solvents, the amount of water found was 36.9 mg.—about 10% too high.

ACKNOWLEDGMENT

The financial support given by Statens Naturvetenskapliga Forskningsråd is gratefully acknowledged. The author also wishes to thank Karin Lindgren and Eivor Ljungqvist for their experimental assistance.

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Spectrophotometric Study of Modified Heteropoly Blue Method for Phosphorus

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A spectrophotometric study has been made of the modified heteropoly blue method in which the yellow molybdophosphoric acid is extracted with isobutyl alcohol and subsequently reduced with chlorostannous acid to the heteropoly blue. The absorption spectrum of the heteropoly blue of phosphorus in isobutyl alcohol is different from that obtained in aqueous medium; characteristic absorbance maxima are found at 625 and 725 mμ. The system conforms to Beer's law with an optimum concentration range from 0.1 to 1.3 p.p.m. of phosphorus when measurements are made at 725 mμ in 1-cm. cells. The effect of solution variables, especially diverse ions, was investigated. The interference from arsenic and germanium can be eliminated by a preliminary volatilization as the bromides. The modified method has been applied to the determination of phosphorus in plain carbon steels.

THE heteropoly acid of phosphorus, in both the unreduced and reduced form, is commonly utilized to determine small amounts of phosphorus. Some of the more commonly occurring elements which also form similar heteropoly acids are arsenic, silicon, and germanium.

In an effort to eliminate interferences and make the heteropoly method more specific, selective extraction of the heteropoly acids has been utilized. Most of the early work on liquid-liquid extraction of heteropoly acids has been summarized by Wadelin and Mellon (*10*).

Berenblum and Chain (*2*) extracted the yellow molybdophosphoric acid with isobutyl alcohol and reduced it to the blue heteropoly complex by shaking the extract with a solution of chlorostannous acid. Allen (*1*) found this method useful for the determination of phosphorus in highly colored or turbid solutions. Schaffer, Fong, and Kirk (*9*) varied the procedure by extracting with n-octyl alcohol and thus determined micro and submicro amounts of phosphorus in biological samples. Pons and Guthrie applied the method to the determination of phosphorus in plant materials (*8*).

Because the method as reported by Berenblum and Chain was not sensitive to variations in acidity and reductant concentration—conditions which ordinarily must be rigidly controlled—further investigation of this method seemed desirable.

This spectrophotometric study was undertaken to determine the effect of solution variables, especially diverse ions, and to apply the modified method to the determination of phosphorus in steel and water samples.

APPARATUS AND REAGENTS

Absorbance measurements were made in 1.000-cm. matched cells with a Beckman Model DU spectrophotometer. The initial spectrophotometric curves were obtained with a Warren Spectracord. A reagent blank was used in the reference cell. However, in most cases the reagent blank did not differ in absorbance from the pure solvent.

The following reagent solutions were prepared.

Standard phosphate solution (0.025 mg. of phosphorus per ml.). Dissolve 0.1098 gram of reagent grade potassium dihydrogen phosphate in distilled water and dilute to 1 liter.

Sodium molybdate solution, 10%. Dissolve 25 grams of sodium molybdate, $Na_2MoO_4 \cdot 2H_2O$, in distilled water and dilute to 250 ml. The solution must be clear.

Chlorostannous acid solution, 0.2%. Dissolve 2.38 grams of stannous chloride, $SnCl_2 \cdot 2H_2O$, in 170 ml. of concentrated hydrochloric acid and dilute to 1 liter with distilled water. Add several pellets of metallic tin.

The isobutyl alcohol used for extraction was Matheson Co., Inc., No. 2858. The perchloric acid used was 72% double vacuum distilled (G. F. Smith Chemical Co.). All other chemicals and acids used were of reagent grade, with the exception of several salts used in the diverse ion study.

All of the aqueous solutions were stored in polyethylene bottles to prevent contamination from silica.

EFFECT OF SOLUTION VARIABLES

The reduction of the yellow heteropoly molybdophosphoric acid, $H_3P(Mo_3O_{10})_4$, to give the familiar heteropoly blue complex must be conducted under controlled conditions (*3, 12*). An excess of molybdate reagent must be present in order to shift the equilibrium to the heteropoly acid side (*10*). This excess reagent is reduced to a blue color if the reduction conditions are not carefully regulated. The heteropoly acid can be separated from essentially all of the excess molybdate reagent by extraction, after which it can be reduced by a strong reductant without any special precautions.

The following procedure was used to study the effect of solution variables upon the formation of molybdophosphoric acid and its extraction with isobutyl alcohol.

A definite amount of the standard phosphate solution was transferred to a 50-ml. volumetric flask and the desired amount of perchloric acid added. In the study of diverse ions, a definite amount of solution containing each diverse ion was also added. The solution was diluted to approximately 40 ml. and 5 ml. of the molybdate reagent was added. The solution was diluted to the mark with distilled water, mixed, and allowed to stand for approximately 5 minutes. It was then transferred to a 150-ml. separatory funnel and extracted with 40 ml. of isobutyl alcohol. The extract was washed twice with 25-ml. portions of distilled water. The aqueous phase was removed and the desired amount of chlorostannous acid added. After shaking, the nonaqueous phase was drained into a 50-ml. volumetric flask. The funnel was rinsed with a small portion of isobutyl alcohol and the washings were drained into the volumetric flask. The solution was diluted to the mark with pure solvent and thoroughly mixed. The visible absorption spectra were recorded, or the absorbance was measured at 725 mμ using a reagent blank solution in the reference cell.

Acidity. The optimum acidity for the formation of molybdophosphoric acid formation has been shown by Boltz and Mellon (*4*) to be approximately 0.3*N*. Berenblum and Chain (*2*) selected an acidity of 0.5*N* and varied it from 0.05*N* to 1.5*N* without any effect on the ultimate color intensity.

Large amounts of iron(III) formed a precipitate with the molybdate reagent in solutions of low acidity. This precipitate did not form at higher acidities. Because it was desirable to eliminate the iron(III) interference, higher acidities were used.

When 0.5 p.p.m. of phosphorus was used with the final acidities in respect to perchloric acid as shown, the following absorbances were obtained:

N, $HClO_4$	0.47	0.7	0.93	1.15	1.4	1.86
Absorbance	0.357	0.357	0.356	0.356	0.358	0.343

A variation in acidity from 0.5*N* to 1.4*N* does not affect the color intensity; therefore, an intermediate value of 1.2*N* was selected for subsequent experimental work.

Molybdate Concentration. A large excess of molybdate ion is necessary for heteropoly acid formation (*10*). With solutions containing 0.5 p.p.m. of phosphorus and 3, 5, and 7 ml. of the molybdate reagent, absorbance values of 0.357, 0.353, and 0.356, respectively, were obtained. Five milliliters of the 10% sodium molybdate solution in a final volume of 50 ml. was selected as a suitable excess.

Extractant. Isobutyl alcohol quantitatively extracts the yellow molybdophosphoric acid. One extraction with approximately 40 ml. of isobutyl alcohol is sufficient to extract the amounts of molybdophosphoric acid used in this study. Two extractions with 20-ml. portions of isobutyl alcohol do not increase the amount of molybdophosphoric acid extracted. Absorbance readings of 0.354 and 0.351, respectively, were obtained at 725 mμ for 0.5 p.p.m. of phosphorus with one 40-ml. extraction and two 20-ml. extractions. Butyl alcohol and presumably other immiscible alcohols could also be used.

Reductant. If the acidity of chlorostannous acid is too low, reduction to the heteropoly blue does not occur. If the acidity is too high, the intensity of the blue color is decreased. Twenty-five milliliters of a 0.2% chlorostannous acid solution, 2*N* in hydrochloric acid, was found to be satisfactory for reduction of the amounts of molybdophosphoric used in this study. This concentration is similar to that used by Berenblum and Chain (*2*).

The volume of reductant may vary from 15 to 35 ml. without affecting the final color intensity. Absorbance values of 0.353 and 0.357 were obtained with 0.5 p.p.m. of phosphorus when 15 and 35 ml. of chlorostannous acid reagent were used.

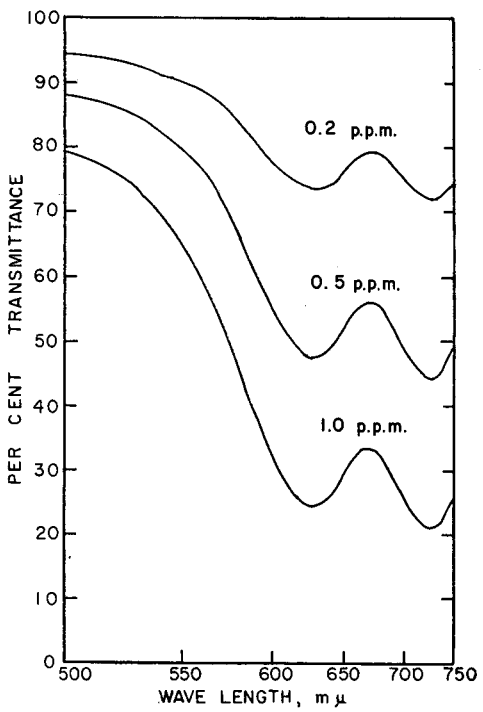

Figure 1. Absorption spectra of heteropoly blue of phosphorus in isobutyl alcohol

Color Development. The time allowed for complex formation before extraction is not critical. With no diverse ions added, identical absorbance values were obtained when the molybdophosphoric acid solutions were allowed to stand 3 minutes and 30 minutes after the addition of molybdate reagent before being extracted.

Stability. The blue color is stable for at least 20 minutes provided the solution is kept in a stoppered flask. There is a slight but constant decrease in color intensity after 20 minutes; the amount of fading after 1 hour is approximately 3%. When 0.5 p.p.m. of phosphorus was used, the following absorbance values were obtained:

Time, minutes	5	10	20	30	45	60	90
Absorbance	0.359	0.359	0.358	0.356	0.353	0.350	0.344

Phosphorus Concentration. The absorption spectra for various concentrations of phosphorus are shown in Figure 1. Beer's law applies at 625 and 725 mμ, with greater sensitivity being obtained by making spectrophotometric measurements at 725 mμ.

The absorption spectrum for the heteropoly blue complex obtained in aqueous solution with a different reductant and different conditions of reduction has its absorbance maximum at 830 mμ with an inflection point at 650 mμ (*3*).

The molar absorptivity for phosphorus at 725 mμ is 22,700

liter per mole centimeter, and at 625 mμ 19,200 liter per mole centimeter.

Effect of Diverse Ions. These studies were made with solutions containing 0.6 p.p.m. of phosphorus. An error of less than 2% of the phosphorus present was considered negligible.

One thousand parts per million (50 mg.) of the following ions did not interfere. (The concentration of diverse ion is expressed as parts per million in solution prior to extraction).

Acetate, bromide, carbonate, chloride, citrate, dichromate, fluoride, iodate, nitrate, nitrite, oxalate, permanganate, sulfate, ammonium, aluminum, barium, bismuth(III), cadmium, calcium, chromium(III), cobalt(II), copper(II), iron(II), iron(III), lead(II), lithium, magnesium, manganese(II), nickel(II), potassium, silver, sodium, thorium(IV), uranyl, and zinc.

When 50 mg. of nitrite was present, 50 ml. of the chlorostannous acid solution were required to reduce the molybdophosphoric acid.

The ions which were found to interfere are listed in Table I, along with the tolerance for adherence to control limits. The tolerance with respect to a diverse ion is often much lower in solutions of higher ionic strength.

Table I. Interfering Ions

Ion	Form Added	Amount Added, P.P.M.	% Error	Tolerance,[a] P.P.M.
As^{+++}	Na_2HAsO_2	100	+15	60
As^{+5}	Na_2HAsO_4	20	Over 100	0
Ce^{+4}	$(NH_4)_2Ce(NO_3)_6$	10	−50	0
Ge^{+4}	Na_2GeO_3	20	Over 100	0
Au^{+++}	$AuCl_3$	20	−20	0
I^-	NaI	1000	−12	60
Hg^+	$HgNO_3$	600	− 6	20
Hg^+	$HgSO_4$	1000	−10	300
Si^{+4}	Na_2SiO_3	500	+25	30
SCN^-	KSCN	1000	−35	60
$S_2O_3^{--}$	$Na_2S_2O_3$	20	− 5	0
Sn^{++}	$SnCl_2$	20	−32	0
Sn^{+4}	$SnCl_4$	1000	−10	40
WO_4^{--}	Na_2WO_4	5	− 8	0
V^{+5}	Na_3VO_4	20	Time variable	0

[a] Causing less than 2% error using 0.6 p.p.m. of phosphorus.

GENERAL PROCEDURES

A suitable sample solution or aliquot, after proper preliminary treatment, should contain 0.01 to 0.06 mg. of phosphorus as orthophosphate. If nitric acid is used to dissolve the sample, or if sodium bromide has been used to remove arsenic or germanium (as described later), add 5 ml. of perchloric acid and evaporate until dense fumes are evolved.

Add 5 ml. of perchloric acid to the solution. Precise duplication of acidity is not required; hence, exact neutrality of the original solution is unnecessary. Dilute to approximately 45 ml. and add 5 ml. of molybdate reagent. Mix and allow to stand for several minutes. Transfer to a glass-stoppered 125-ml. separatory funnel. Rinse the vessel with a small portion of distilled water and add the washings to the separatory funnel. Extract with 40 ml. of isobutyl alcohol by shaking for 60 seconds. Drain and discard the lower aqueous layer. Shake the isobutyl alcohol extract with two successive 25-ml. portions of distilled water. Discard the lower aqueous layers. Swirl the solution in the funnel to collect droplets of water into one globule and discard.

Add 25 ml. of chlorostannous acid reagent and shake for 15 seconds. Discard the lower aqueous layer and drain the alcohol phase into a 50-ml. volumetric flask. Wash the funnel with 10 ml. of isobutyl alcohol and add the washings to the 50-ml. volumetric flask. Dilute to the mark with isobutyl alcohol. The solution should be a clear blue. After mixing thoroughly, measure the absorbance at 725 mμ using a reagent blank as reference. Determine the amount of phosphorus present by referring the absorbance reading to a standard curve obtained under the same conditions from standard phosphate solutions.

Elimination of Arsenic and Germanium Interference. The interference caused by germanium and arsenic can be eliminated by volatilizing these elements in the form of the proper halide (*5–7, 11*). Arsenic is best removed as the bromide. Both quinquevalent and trivalent arsenic were distilled from a perchloric acid solution after the addition of sodium bromide. Five milliliters of 35% aqueous sodium bromide solution are sufficient for quantitative removal of at least 25 mg. of arsenic. Germanium is readily volatilized either as the bromide or the chloride. Twenty-five milligrams of germanium is quantitatively removed by the addition of 5 ml. of 35% sodium bromide solution and subsequent evaporation to perchloric acid fumes.

Thus, by the addition of sodium bromide the interference caused by germanium and arsenic is eliminated in one operation. The silicon present is also made innocuous in the same operation when the solution is evaporated to perchloric acid fumes. It is not necessary to remove the dehydrated silica by filtration because in the extraction process the precipitate remains in the aqueous layer.

The germanium can also be removed by adding 10 ml. of concentrated hydrochloric acid and heating. This treatment quantitatively removes up to 25 mg. of germanium. However, arsenic(III) and arsenic(V) are not completely volatilized, and are best removed as the bromides.

The following procedure was used for the removal of arsenic and germanium from samples containing small amounts of iron.

Add 5 ml. of 72% perchloric acid to the solution of the sample. Add 5 ml. of 35% aqueous sodium bromide and evaporate to dense fumes of perchloric acid. It is important that the solution be evaporated to dense fumes in order to ensure complete elimination of the interfering ion. Cool the solution, add approximately 40 ml. of water, and determine the phosphorus as just described

No loss in phosphorus was detected using this step with up to 25 mg. of arsenic and germanium.

PROCEDURE FOR PLAIN CARBON STEELS

Adjust the sample size so that the amount of phosphorus is within the limits of this method (0.01 to 0.06 mg.). Weigh the steel sample and transfer to a conical flask. Dissolve the sample with 3 ml. of nitric acid and 5 ml. of hydrochloric acid. The amounts may be varied according to sample size. Add 6 ml. of 72% perchloric acid and evaporate to dense perchloric acid fumes. Fuming removes nitric acid which, if present, would oxidize the bromide. Cool, wash the sides of the flask, and add at least 5 ml. of 35% sodium bromide solution for every 15 mg. of arsenic present in the sample. Continue heating until the dark brown ferric bromide complex decomposes, liberating bromine. Again fume for several minutes. Cool, add approximately 40 ml. of water, and determine the phosphorus according to the general procedure.

Table II. Determination of Phosphorus in Standard Steels

Type of Steel	NBS No.	Phosphorus, % Certificate value	Found	Diff.
BOH	14*c*	0.012	0.011	−0.001
BOH	11*d*	0.006	0.0066	+0.0006
BOH	13*d*	0.016	0.014	−0.002
AOH	21*c*	0.062	0.060	−0.002
AOH	35*a*	0.037	0.035	−0.002
Bessemer	10*d*	0.088	0.083	−0.005
High silicon	125	0.008	0.005	−0.003
Cr-Mo	72*d*	0.017	0.014	−0.003
Mo-Ni	111*a*	0.017	0.014	−0.003

Inasmuch as the precise duplication of acidity is not necessary for reliable results, the quantity of perchloric acid used in dissolving the sample or in oxidizing the excess sodium bromide is not a critical solution variable.

Germanium is not completely volatilized from a steel sample as either the bromide or chloride when the above procedure is used. Presumably, the volatilization of germanium halides is inhibited by the high concentration of iron(III) halide complexes. However, up to 1 mg. of germanium may be present in the steel without causing interference if phosphorus is deter-

mined according to the general procedure. The addition of 20 ml. of hydrochloric acid and 6 ml. of perchloric acid after dissolution of the sample and heating to perchloric acid fumes removes 5 mg. of germanium. When a larger amount of germanium is present, prolonged fuming leaves a residue which dissolves with difficulty.

Table II lists the results when the recommended procedure was used to determine the phosphorus content of several plain carbon steel samples.

The results listed in Table II are the average of two determinations with a maximum deviation of 0.002%. In general the experimental values are slightly below the certified value. For steel samples containing no arsenic, higher results were obtained when the addition of sodium bromide was omitted. However, no loss of phosphorus was observed when sodium bromide was used on synthetic samples.

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RECEIVED for review October 12, 1955. Accepted April 16, 1956. Division of Analytical Chemistry, 128th meeting, ACS, Minneapolis, Minn., September 1955.

Interpretation of 10.3-Micron Infrared Absorption Band in Lubricating Oils

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Infrared absorption by lubricating oils in the 10.3-micron region is due to two different types of structural groups. *trans*-Olefins produce a band at 10.35 microns which is removed by hydrogenation and is not concentrated by thermal diffusion. Another type of structure, probably polycyclic naphthenes, produces a band at 10.27 microns which is not removed by hydrogenation and is concentrated in the bottom fractions by thermal diffusion.

THE work of Fred and Putscher (*1*, *5*) and Haak and van Nes (*2*) strongly indicates that olefinic structural groups present in Pennsylvania oils produce an infrared absorption band near 10.3 microns. However, Lillard, Jones, and Anderson (*4*) have shown that absorption in the 10.3-micron region is not due solely to olefinic groups, but may be produced by other structures, of which polycyclic naphthenes are most likely. The work reported here confirms these previous results and, in addition, indicates that these two types of structural groups, when present in lubricating oils, can be distinguished by means of the infrared spectrum. It is concluded here that *trans*-olefinic groups produce an absorption band at 10.35 microns and that some other structure, probably associated with polycyclic naphthenes, produces a band at 10.27 microns. Evidence for this conclusion is that the 10.35-micron band is removed by hydrogenation and is not concentrated appreciably by thermal diffusion, while the 10.27-micron band is not removed by hydrogenation and is concentrated in the bottom fractions from a thermal diffusion column.

EXPERIMENTAL

Results are reported here for two oil samples. Oil A was a fraction obtained from the silica gel percolation of a Mid-Continent distillate. It was collected near the end of the paraffin-naphthene portion, was essentially free of aromatics, and had an enhanced absorption in the 10.3-micron region. Spectra in the 10.3-micron region are shown in Figure 1,*a*, before and after hydrogenation. The original oil has two bands of about the same intensity at 10.27 and 10.35 microns. Hydrogenation preferentially reduced the intensity of the 10.35-micron band, thus indicating that the 10.35-micron band is associated with olefinic groups and that the 10.27-micron band is associated with some other structure. Another sample similar to oil A was separated into 10% cuts by thermal diffusion. Figure 1,*b*, shows spectra of two of these cuts. The 0 to 10% cut from the top of the column shows only the 10.35-micron band. The 80 to 90% cut from near the bottom of the column has its strongest band at 10.27 microns and a shoulder near 10.35 microns. This result suggests that the 10.27-micron band is associated with polycyclic naphthenes,

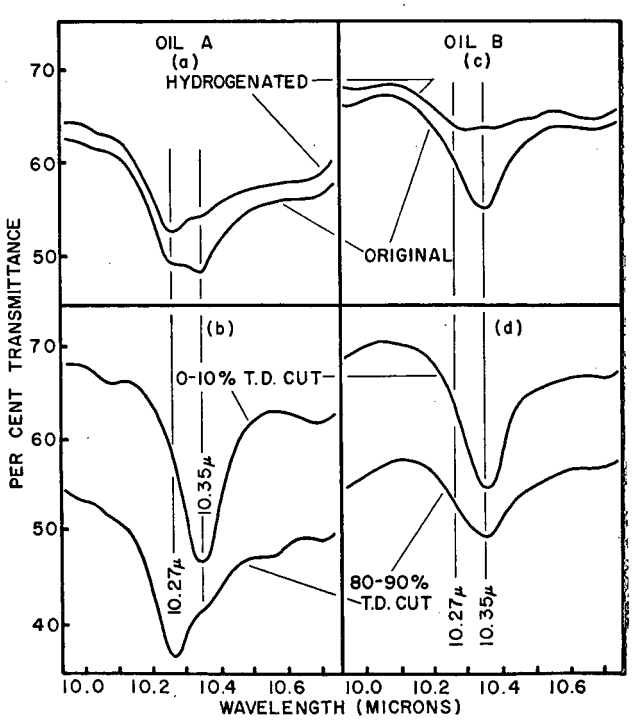

Figure 1. Infrared spectra of oil fractions

because these molecules are known to concentrate at the bottom of a thermal diffusion column (*3*).

Oil B was a refined and dewaxed residual oil and was studied without additional fractionation. The original oil had a band at 10.35 microns and no indication of a band at 10.27 microns as shown in Figure 1,*c*. Hydrogenation removed this band almost completely. Spectra of two of the thermal diffusion cuts are shown in Figure 1,*d*. There is some broadening of the 10.35-micron band on the short wave length side in the 80 to 90% cut from near the bottom of the column, but it appears that the band in this oil is due primarily to *trans*-olefin structural groups.

ACKNOWLEDGMENT

The author wishes to express his thanks to E. R. Kerr who carried out the hydrogenations, and to J. E. Scardefield who helped with the experimental work.

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RECEIVED for review December 19, 1955. Accepted February 24, 1956.

Modified Combustion Procedure for Determining Carbon and Hydrogen in Certain Organometallic Compounds

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A combustion analysis apparatus and procedure are described which permit the determination of carbon and hydrogen in volatile pyrophoric compounds which may undergo pyrolysis to give nonvolatile residues.

IN THE course of an investigation of hydrides and organometallic compounds of magnesium and beryllium it became necessary to use combustion analysis to determine the ether content and composition of certain preparations. The properties of these compounds made it impossible to use the conventional combustion procedure without modification. Several of the compounds were pyrophoric, others were very volatile, and some released hydrogen suddenly and in such copious quantities that, in an oxygen atmosphere, explosive mixtures resulted. An additional difficulty arose from the pyrolysis of some of the preparations and the formation of a nonvolatile residue which required exposure to oxygen at an elevated temperature for complete combustion.

A modification of the conventional combustion procedure was developed and used successfully on these preparations.

APPARATUS

The apparatus as finally developed consisted of a modified Liebig combustion train containing elements in sequence as follows:

Two parallel butyl sebacate bubblers for the admission of oxygen and helium.

A quartz tube (15 mm. in outside diameter × 20 cm. long) containing copper oxide maintained at 700° C. for purification of inlet gases.

A U-tube containing successive sections of Anhydrone (G. Frederick Smith Chemical Co., Columbus, Ohio), Ascarite (Arthur H. Thomas Co., Philadelphia, Pa.), and Anhydrone for removal of water and carbon dioxide from inlet gases.

A quartz sample tube (15 mm. in outside diameter × 18 cm. long) and accompanying furnace.

A quartz combustion tube (23 mm. in outside diameter × 34 cm. long) filled with copper oxide and maintained at 900° C. to assure combustion of carbon monoxide (*3*).

Two U-tubes fitted with stopcocks and containing Anhydrone for absorption of water, followed by two additional tubes each containing Ascarite followed by Anhydrone in approximately equal amounts for absorption of carbon dioxide.

A final bubbler for observation in adjusting gaseous flow rates.

All connections at critical locations were made with semiball joints, and flexible tubing was excluded from these locations. The flow rate of gas through the system was maintained at approximately 2.5 liters per hour. This flow rate was maintained between runs to assure steady-state conditions. All stopcocks and the first ball joint on the exit side of the combustion furnace were greased with Apiezon N. This ball joint was greased to prevent any loss of the water condensing at this point. Grease was not necessary on the other ball joints.

PROCEDURE

Because of the highly reactive materials handled—for example, dimethylberyllium, magnesium hydride, aluminum hydride, and beryllium hydride—some satisfactory method was needed whereby the material could be easily weighed and placed into the combustion system. This was accomplished by using a small tin capsule which could be introduced with the sample and completely oxidized in the course of the combustion. These capsules were made from pieces of tin 4 cm. square, about 3 mils thick, and weighing approximately 0.6 gram. After a sheet had been rolled on a mandril, the side and one end were sealed by crimping. The open capsule was placed in a weighing bottle, taken into a drybox, filled with the inert gas of the box, taken out, and weighed using another bottle as a tare. The capsule and bottle were taken back into the drybox; the capsule was loaded, sealed by crimping, taken out of the drybox, and reweighed. Samples of 50 mg. were found to be a convenient size.

After weighing, the capsule containing the sample was placed quickly in a quartz boat which had been fired previously in a muffle furnace at 1000° C. for at least 1 hour. The boat containing the sample was introduced through a semiball joint immediately into the sample-furnace region. Just prior to this operation the absorption train had been attached to the system, the stopcocks opened, and the flow of helium begun. Because of the rapid release of hydrogen and other explosive gases from some of these materials during combustion, it was necessary to use pure helium during the initial stage of heating. After the more volatile portions of the sample had had time to pass through the combustion furnace, the flow of oxygen was begun. Under these conditions one sample could be run about every $2^1/_2$ hours.

After the sample had been placed in position and the system closed, the furnace surrounding the sample tube was turned on at its maximum power of 800 watts until the maximum operating temperature of about 1050° C. was reached. This high temperature was used to destroy any stable carbonates which might have been formed during the initial heating of the sample. At a sample temperature of about 600° C. the helium was stopped; pure oxygen was then admitted and continued for the remainder of the run. It was noted that a large consumption of oxygen occurred between approximately 850° and 900° C. due to the burning of the tin. For this reason the flow of oxygen into the system had to be increased to maintain the rate of 2.5 liters per hour through the exit bubbler. The temperature of 1050° C. was reached in 25 minutes and was maintained until the visible moisture on the exit side of the combustion furnace had disappeared. At this

time the run was terminated. The amounts of carbon and hydrogen were obtained in the usual manner from the weight gains of the corresponding absorption tubes.

DETERMINATION OF BLANK CORRECTION

Nine blank runs (Table I) were made with the collecting train in position and the combustion and purification furnaces at their operating temperatures. The nine runs include those made with freshly charged tubes and also after carbon dioxide and water from a combustion had been absorbed. Under these conditions no distinguishable differences were noted.

The weight gain or loss per hour of run was determined, from which the mean and the standard deviation of the mean were determined. The uncertainty given is 2 × the standard deviation. The blank for carbon dioxide was found to be 0.030 ± 0.016 mg. per hour and for water −0.005 ± 0.012 mg. per hour. Because a run usually lasted no more than 2 hours, a blank correction was thus found to be unnecessary.

Combustion of the tin used showed that it gave 0.22 mg. of water per gram of tin and 0.03 mg. of carbon dioxide per gram of tin.

COMBUSTION OF STANDARD SAMPLE

Succinic acid, Eastman Kodak Co., white label purity, was burned to determine the efficiency of the combustion apparatus. The acid was dried for more than 2 hours in an oven at 110° C. The results are shown in Table II.

Table I. Evaluation of Blank Correction for Carbon Dioxide and Water

Time of Run, Hours t	Wt. Gain, Mg.	Gain, Mg./Hour Carbon Dioxide	Deviation, Δ	$t \times \Delta^2$
1.5	0.2	0.13	0.10	0.0150
1.0	0.0	0.00	0.03	0.0009
1.5	0.0	0.00	0.03	0.0014
1.7	−0.2	−0.12	0.15	0.0382
1.7	0.0	0.00	0.03	0.0015
10.0	0.3	0.03	0.00	0.0000
2.0	0.1	0.05	0.02	0.0008
2.0	0.3	0.15	0.12	0.0288
15.0	0.4	0.03	0.00	0.0000
36.4				0.0866
		Water		
1.5	0.1	0.07	0.08	0.0096
1.0	−0.2	−0.2	0.19	0.0361
1.5	0.0	0.0	0.01	0.0002
1.7	0.0	0.0	0.01	0.0002
1.7	0.0	0.0	0.01	0.0002
10.0	−0.1	−0.01	0.00	0.0000
2.0	0.0	0.0	0.01	0.0002
2.0	0.0	0.0	0.01	0.0002
15.0	0.0	0.0	0.01	0.0015
36.4				0.0482

Av. wt. gain per hour: For CO_2 = 0.030 mg.
For H_2O = −0.005 mg.

Std. dev. from mean of single observation = $\sigma = \sqrt{\frac{\Sigma(t \times \Delta^2)}{\Sigma t - 1}}$

For CO_2 = $\sqrt{\frac{0.0866}{35.4}}$ = 0.0495

For H_2O = $\sqrt{\frac{0.0482}{35.4}}$ = 0.0369

2 × std. dev. of mean = $\frac{2\sigma}{\sqrt{\Sigma t}}$

For CO_2 = $\frac{2 \times 0.0495}{\sqrt{36.4}}$ = ±0.0164

For H_2O = $\frac{2 \times 0.0369}{\sqrt{36.4}}$ = ±0.0122

Blank correction: For CO_2 = 0.030 ± 0.016 mg./hour
For H_2O = −0.005 ± 0.012 mg./hour

Table II. Combustion of Standard Succinic Acid

Sample, Mg.	Carbon and Hydrogen Determination: H_2O found, mg.	Hydrogen, % Calcd.	Hydrogen, % Found	Ratio, Found/Calcd.
205.1 ± 0.2	94.2 ± 0.24	5.12	5.14 ± 0.014	1.0039 ± 0.0027
62.3 ± 0.2	28.4 ± 0.24	5.12	5.10 ± 0.046	0.9961 ± 0.0090
	CO_2 Found, Mg.	Carbon, % Calcd.	Carbon, % Found	
205.1 ± 0.2	304.9 ± 0.30	40.68	40.57 ± 0.06	0.9973 ± 0.0015
62.3 ± 0.2	91.2 ± 0.30	40.68	39.95 ± 0.19	0.9821 ± 0.0047

EXAMPLES AND DISCUSSION

The materials burned in the apparatus were mainly hydrides and organometallic compounds of beryllium and magnesium containing ether and other contaminants. The materials included:

Beryllium hydride
Beryllium borohydride
Dimethylberyllium
Methylberyllium hydride
Aluminum hydride
Aluminum borohydride
Lithium aluminum hydride
Magnesium hydride
Diethylmagnesium

Typical results from three different runs are given in Table III. The data obtained from an independent hydrolysis of the material are given, followed by the data obtained from a combustion of the same material. The blank spaces indicate either the absence of a substance in the sample or else the impossibility of obtaining those data.

In the hydrolysis of dimethylberyllium the amount of methane found by mass spectrographic analysis of the gas was broken down into hydrogen and carbon in the ratio of 3 to 1 for inclusion in the table. In the combustion of dimethylberyllium the amounts of hydrogen and carbon collected as water and carbon dioxide, respectively, were in the ratio of 3.00 to 1, indicating good precision for the run. Other than for compounds containing organic groups, the direct comparison between hydrolysis carbon and combustion carbon means little. However, these values were necessary in order to obtain the data for a direct comparison between the hydrogen results of the two methods. For example, the beryllium hydride compounds frequently had appreciable amounts of dimethylberyllium present in addition to diethyl ether. For this reason the per cent of methane evolved from the sample during hydrolysis had to be measured so that a correction could be applied to the combustion carbon results to give a measure of the ethyl ether content of the sample. This, in turn, permitted an evaluation of the hydrogen balance and the calculation of the amount of hydride hydrogen present in the sample.

Table III. Comparison of Combustion and Hydrolysis Analyses

Material	Sample Wt., Mg.	Carbon Source	Carbon Mg.	Carbon %	Hydrogen Source	Hydrogen Mg.	Hydrogen %
Me_2Be (hyd.)	77.8		46.83	60.19		11.71	15.05
Me_2Be (comb.)	46.3		28.26	61.04		7.07	15.27[a]
AlH_3 (hyd.)	50.7				AlH_3	2.97	5.86
AlH_3 (comb.)	47.4				AlH_3	2.74	5.78
		Ether	10.37	21.85	Ether	2.16	4.56
BeH_2 (hyd.)	44.8				BeH_2	5.92	13.21
		Me_2Be	0.18	0.40	Me_2Be	0.045	0.10
BeH_2 (comb.)	33.4				BeH_2	4.48	13.41
		Me_2Be	0.13	0.39	Me_2Be	0.03	0.09
		Ether	0.79	2.38	Ether	0.17	0.51

[a] Hydrogen-carbon atom ratio for this sample was 3.00 to 1 and in agreement with expected ratio.

The completeness of combustion and the over-all behavior of the apparatus were tested and proved satisfactory by burning succinic acid as a standard. There remained, however, the question of whether a relatively large quantity of ether, as was liberated from such materials as beryllium hydride etherate, would be burned completely or whether only partial combustion would occur upon its passage through the combustion furnace. This was tested by burning a sample of purified ether in the apparatus. The ether was introduced from a sealed tube equipped with a breakoff seal, which was broken after the tube had been sealed into the system. The ether was then vaporized and carried into the combustion region by means of helium passing over the opening of the tube. By this method, a sample of ether weighing 63.3 mg. gave 99.1% of the expected amount of carbon dioxide and 99.3% of the expected amount of water.

Also, completeness of combustion was indicated when the amount of active hydrogen determined by hydrolysis checked with that found by combustion on compounds where this comparison was possible.

Some workers (*4*) have found that successive cycles of heating a quartz tube above 1100° C. with subsequent cooling cause porosity in the quartz, which might eventually develop into leaks in the tube. Also, the use of copper oxide in quartz at high temperatures tends to weaken the quartz eventually to the point where a slight strain will cause it to crack.

PRECISION AND ERROR

The absorbents used were Ascarite and Anhydrone. It has been reported (*1*) that Ascarite will absorb carbon dioxide completely at a flow rate as high as 0.5 liter per minute until its weight has increased about 20%. Anhydrone has been reported (*2*) to be as good a desiccant as phosphorus pentoxide at a maximum flow rate of about 5 liters of gas per hour and absorbs up to 60% of its weight of water.

Even though an initial copper oxide furnace and an absorption tube containing Anhydrone and Ascarite were used, a small blank was found, as has been shown. Because the blank corrections were negligible, they were not made on the runs; however, they were included in calculating the per cent error.

The weighings were carried out on an Ainsworth Chainomatic balance (Wm. Ainsworth and Sons, Denver, Colo.). An attempt was made to estimate the weights to a few hundredths of a milligram so that their reproducibility was within the limits of ±0.1 mg. Hence, for each weight recorded a possible error of ±0.2 mg. was allowed (because weighings were made by difference). In addition to the weighing uncertainty, an allowance was made for the carbon dioxide and water blanks, 0.05 and 0.02 mg. per hour, respectively, including the uncertainty in the blanks. The per cent errors in the carbon dioxide and water yields were figured separately. The total uncertainty (in per cent) for a given determination was taken as the square root of the sum of the squares of the uncertainties in the weight of the product—i.e., carbon dioxide or water—and in the original sample weight.

From these considerations it is possible to predict the expected precision for a combustion of any sample of given size. Because different substances yield varying amounts of carbon dioxide and water, a specific sample size which would ensure a certain per cent accuracy cannot be given. The sample size should be so chosen that the per cent uncertainty for the product of lowest yield is within the desired limits.

ACKNOWLEDGMENT

Acknowledgment is made to T. W. Newton and J. F. Lemons for their helpful suggestions concerning this work.

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RECEIVED for review January 27, 1956. Accepted April 4, 1956. Work done under the auspices of the U. S. Atomic Energy Commission.

Assay for Platinum Metals in Ores and Concentrates

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There are no data recorded to indicate the precision achieved by the various methods used for the fire assay of platinum ores. In the following report platinum metals ores and concentrates have been examined by direct assaying and by methods involving leaching prior to fire assay. The silver–platinum metals beads were examined spectrographically. The data obtained suggest that for ores and concentrates no advantage is to be gained by the elaborate, time-consuming leaching methods.

IT IS generally recognized that there is an appreciable lack of precision in platinum metal values obtained from various laboratories which use different methods of fire assay. Experience has shown that the numbers vary by as much as a factor of 10. These discrepancies may be due in part to variations in procedure and technique.

Leaching processes were developed many years ago and are still used by some analysts as a preliminary treatment before fire assay. This process converts the bulk of the minerals to simple dissolved constituents and with subsequent treatment there is some isolation of base metals. There is no published evidence that better results are obtained by these time-consuming procedures.

A unique opportunity was presented to evaluate the efficiencies of fire assay and leaching practices by an invitation to take part in a reconnaissance survey. The fire assay for platinum (*2*) was being investigated in the authors' laboratory and it was considered desirable to obtain some information regarding losses with assays of ores.

APPARATUS, REAGENTS, AND ORES

A pyrometrically controlled Williams and Wilson 15-kva. Globar-type assay furnace was used for all the fire treatments.

Spectrographic examinations of the silver beads were made on an Applied Research Laboratory 2-meter grating spectrograph (36,600 lines per inch).

Zinc metal dust, Purple Seal grade, obtained from City Chemical Co., New York, N. Y., was used.

Litharge, soda ash, borax glass, and calcium oxide, used in the

[1] Falconbridge Metallurgical Laboratories, Richvale, Ontario, Canada.

Table I. Assay Charges for Direct Fire Assay

Sample No.	Litharge, Grams	Soda Ash, Grams	Borax Glass, Grams	Silica Sand, Grams	Flour, Grams	KNO_3, Grams	Button Size, Grams
O-1	100	60	40	22	3.8		29
O-2	100	60	40	22	3.8		28
O-3	100	60	40	22	3.8		28
O-4	100	60	40	22	8.0		68
O-4[a]	65				8.0		51
O-5N	150	80	16			5.4	57
O-6N	150	80	16			12.0	37
O-7N	150	80	16			5.0	58
O-7N[a]	65				8.0		67
P-1	130	50	40	62	4.4		17
P-2	135	50	40	62	7.2		36
P-3	135	50	40	62	5.6		25
P-3[a]	30				5.6		14
P-4	135	50	40	62	12.0		80
P-4[a]	60				8.4		67
P-5N	150	50	10	30		26	101
P-5N[a]	50				6.0		51
P-6N	175	40	10	60		50	30
P-6N[a]	30				3.0		19
P-7N	225	40	10	60		50	38
P-8N	225	40	10	60		54	34
P-8N[a]	30				4.2		31
H-1	316	30		13.2	3.3		27
H-2	316	30		13.2	3.3		29
H-2[a]	30				3.3		23
H-3	340	30		60	3.3		22
H-3[a]	30				4.2		22
H-4	340	30		60	9.0		79
H-4[a]	60				6.5		55
H-5N	250	35		15		42	20
H-6N	250	35		15		39	30
H-6N[a]	30				3.3		31

[a] Reassay of slag.

preparation of fluxes, were either technical or commercial grades. All other chemicals used were of reagent grade. The following three platinum-bearing ore samples were used: plant feed composite, designated O; pyrrhotite concentrate, P; and high grade concentrate, H.

The percentage composition of the samples was determined, with the following results:

Sample	Fe	Cu	Ni	S	Insol.
O	23.10	0.90	1.53	12.63	...
P	55.50	0.08	1.02	46.00	3.36
H	36.30	5.18	4.66	27.75	16.80

SPECTROGRAPHIC METHOD

The method used for quantitative spectrographic analysis of fire assay beads is a modification of the procedure described by Hawley and Rimsaite (*1*). The standards, made with a lead base, contained 10% of silver and concentrations of platinum, palladium, and rhodium varying between 1.0 and 0.001%. Master standards were prepared by adding platinum, palladium, and rhodium (in high concentrations to minimize weighing errors) to granular lead. Each master standard was melted under hydrogen in a graphite crucible. These lead beads were filed with fine-toothed files, a new file being assigned to each standard and used only for that standard.

Weighed amounts of filings from each master standard were diluted with lead to provide a series of lead bead standards with various proportions of platinum, palladium, and rhodium. The constant 10% of silver and the "scrambling" of platinum metals concentrations is intended to minimize possible spectrographic effects of one element on another.

The precision of determinations on standard samples containing 0.001% of platinum, palladium, and rhodium was, respectively, 4, 6, and 15%.

A fire assay bead was weighed and dissolved in sufficient lead so that the final lead bead contained 10% of silver. The lead bead was weighed, sampled, and analyzed spectrographically to find the percentages of platinum, palladium, and rhodium. These percentages were multiplied by the weight of the lead bead to find the number of milligrams of each platinum metal in the fire assay bead.

In Tables III, IV, and V, blank spaces were left in those cases where no platinum, palladium, or rhodium lines appeared in the spectra. These elements probably were present in very low concentration, but were below the limits of detection of the method used.

DIRECT FIRE ASSAY METHOD

Preparation of Assay Charge. For each determination one assay ton (29.166 grams) of sample was placed in a shallow silica dish. All samples, except those fluxed with potassium nitrate (niter assay), were placed in an electric muffle and the temperature was raised to 675° C. The door of the oven was left slightly open to allow easy oxidation and roasting was continued overnight. The contents of the silica dish were transferred to a large cellophane sheet by being passed through a Standard No. 45 sieve, and silver powder (10 mg.) was added. Various amounts of fluxing substances as shown in Table I were sieved before being added and the whole charge was intimately mixed by rolling. In Table I the letter N following a sample type designates a niter assay.

Assay Procedure. The charges were transferred to assay crucibles and fused in the furnace between the temperatures given below:

975–1150 C. for O samples
975–1100 C. for ON samples
975–1150 C. for P samples
975–1100 C. for PN samples
1100–1150 C. for H samples
975–1150 C. for HN samples

The fusion mixtures were poured into iron molds and the lead buttons when cool were broken away from the slags and weighed. In some instances reassays were made on the slag to test whether a better recovery of precious metals could be obtained from the sample. In these cases the slag from an assay was ground to pass a No. 45 standard sieve and mixed with extra litharge, flour, and a little silver powder. This mixture was fused in the original pot and a second lead button was obtained.

Scorification Procedure. The influence of oversize buttons on the recovery of platinum metals was tested in some cases and this resulted in weights of lead beyond the absorptive capacity of the cupels. The combination of assay and reassay buttons for the formation of a single silver bead also resulted in too much lead for a cupel. Reduction in weight of lead was made by heating at 900° C. on scorifying dishes (3 or 4 inches in diameter) for appropriate lengths of time. The fusion mixtures were poured into iron molds, and when cool the lead buttons were separated from the slag (chiefly fused litharge).

For the cupellation of lead buttons, bone ash cupels preheated at 900° C. for at least 10 minutes were used to form the silver–platinum metals bead.

Table II. Direct Fire Assay Spectrographic Analyses of Fire Assay Beads

(Figures represent Troy ounces per ton)

Sample No.	Platinum	Palladium	Rhodium
O-1	0.0048	0.0031	0.0012
O-2	0.0134	0.0033	0.0016
O-3	0.0040	0.0030	0.0015
O-4	0.0047	0.0031	0.0010
O-5N	0.0051	0.0065	0.0059
O-6N	0.0037	0.0027	0.0013
O-7N	0.0032	0.0031	0.0011
P-1	0.0028	0.0023	0.0022
P-2	0.0031	0.0022	0.0017
P-3	0.0033	0.0028	0.0037
P-4	0.0029	0.0043	0.0017
P-5N	0.0048	0.0030	0.0019
P-6N	0.0033	0.0040	0.0023
P-7N	0.0033	0.0027	0.0015
P-8N	0.0033	0.0032	0.0021
H-1	0.0231	0.0146	0.0045
H-2	0.0264	0.0142	0.0041
H-3	0.0207	0.0130	0.0036
H-4	0.0220	0.0156	0.0042
H-5N	0.0209	0.0142	0.0038
H-6N	0.0205	0.0144	0.0034

The results obtained from the spectrographic analysis of fire assay beads obtained by the direct method are shown in Table II. Averages for these results are shown in Tables III, IV, and V.

LEACHING PRIOR TO FIRE ASSAY

Leaching Procedure. One assay ton of each sample was weighed into a shallow silica dish. The sample was roasted overnight at 675° C. in an electric muffle with the door slightly open. The contents of the silica dish were transferred to a 400-ml. beaker and 50 ml. of concentrated hydrochloric acid were added. The beaker and contents were placed on the steam bath and left overnight. Fifty milliliters of water were added and the sample was filtered using a 9.0-cm. Whatman No. 40 filter paper. The residue was washed well with water and the hydrochloric acid treatment was repeated. After a second filtration and washing, the residue and paper were placed in a Coors No. 3 porcelain crucible and ashed in the muffle. It was noticed that during leaching the P sample was greatly reduced in bulk, but its color was unchanged. The H sample was slightly reduced in bulk and appeared bleached. The O sample took on a sandy appearance. These leached residues were assayed and the results are included in Tables III, IV, and V.

Treatment of Filtrate. METHOD A. The filtrates from the hydrochloric acid treatments were combined and evaporated to a volume of about 50 ml. Two hundred milliliters of water were added and the solution was heated to boiling. Hydrogen sulfide gas was bubbled in while the sample was allowed to cool to near room temperature. The precipitate was recovered by filtration on a 9.0-cm. No. 40 filter paper and washed with 1% ammonium chloride solution. The filter paper and contents were placed in a Coors No. 3 porcelain crucible and heated overnight at 675° C. Filtrates from the sulfide precipitates were assayed and revealed only insignificant amounts of platinum metals.

METHOD B. The filtrate was evaporated to dryness and 150 ml. of water were added. The mixture was heated to boiling and enough hydrochloric acid was added to dissolve the residue. Zinc dust was added until all reaction had ceased and about 15 grams were added in excess. The mixture which contained base metal hydroxides and zinc was boiled for 0.5 hour, filtered through a 9.0-cm. No. 40 filter paper, and washed with water. Ashing was done in a large porcelain crucible as before and it was noticed that some zinc was eliminated by volatilization.

Composition of Fluxes. The compositions of the fluxes used were as follows:

Type of Flux	Silica Sand, %	Borax Glass, %	Calcium Oxide, %	Soda Ash, %	Litharge, %
Neutral flux	15.4	7.7	6.4	44.9	25.6
Base metals flux	14.9			7.5	77.6

Preparation of Assay Charge. The residues remaining in the porcelain crucibles after ignition were transferred to a large cellophane sheet by being passed through a U. S. Standard No. 45 sieve. The following additions were made to all samples except the filtrate residues obtained by Method B:

Neutral flux, 80 grams
Litharge, 28 grams
Flour, 2.8 grams
Silver powder, 10 mg.

After being intimately mixed by rolling on the cellophane sheet, the mixture was transferred to an assay crucible and covered with 20 grams of the neutral flux. Filtrate residues obtained by Method B were mixed with 320 grams of the base metals flux, extra litharge, flour, and silver powder. Eighty grams of the flux were used as a cover. Because the amount of residue was small for O samples by Method B, half of the above weights of base metals flux were used.

Fire Assay Procedure. The pots were placed in the furnace at 975° C. and the temperature was raised at the maximum rate to 1200° C. The fusion mixtures were poured into conical iron molds and allowed to cool. The lead button was broken away and any slag adhering to the button was removed by gentle tapping with an iron rod. Where a reassay was made on the slag, it was ground to pass a No. 45 standard sieve and fused in the original pot with flour and extra litharge to replace that lost to the first button. The lead–platinum metals button was cupelled as described above for the direct fire assay method.

Table III. Results of Leaching Experiments

(Figures represent Troy ounces per ton. O sample)

Expt.	Treatment of Filtrate	Platinum	Sum	Palladium	Sum	Rhodium	Sum
1a		0.0058		0.0006		0.0013	
1b	Method A	...	0.0058	0.0006	0.0012	...	0.0013
2a		0.0038		0.0007		0.0014	
2b	Method A	...	0.0038	0.0015	0.0022	...	0.0014
3a		0.0039		...		0.0011	
3b	Method B	...	0.0039	0.0038	0.0038	...	0.0011
4a		0.0046		...		0.0010	
4b	Method B	0.0031	0.0077	0.0031	0.0031	0.0006	0.0016
		Av.	0.0053		0.0023		0.0013
Direct assay		Av.[a]	0.0043 ± 0.0007		0.0030 ± 0.0002		0.0013 ± 0.0002

a. Leached residue.
b. Leach precipitate.
[a] One analysis reported in Table II was deleted.

Table IV. Results of Leaching Experiments

(Figures represent Troy ounces per ton. P sample)

Expt.	Treatment of Filtrate	Platinum	Sum	Palladium	Sum	Rhodium	Sum
1a		0.0011		0.0008		0.0024	
1b	Method A	0.0010	0.0021	0.0015	0.0023	...	0.0024
2a		...		...		0.0014	
2b	Method B	0.0023	0.0023	0.0024	0.0024	0.0009	0.0023
3a		...		...		0.0014	
3b	Method B	0.0040	0.0040	0.0024	0.0024	0.0006	0.0020
4a		0.0014		...		0.0018	
4b	Method B	0.0021	0.0035	0.0017	0.0017	..	0.0018
		Av.	0.0030		0.0022		0.0021
Direct assay		Av.	0.0034 ± 0.0006		0.0031 ± 0.0008		0.0021 ± 0.0007

a. Leached residue.
b. Leach precipitate.

Table V. Results of Leaching Experiments

(Figures represent Troy ounces per ton. H sample)

Expt.	Treatment of Filtrate	Platinum	Sum	Palladium	Sum	Rhodium	Sum
1a		0.0170		0.0067		0.0031	
1b	Method A	0.0010	0.0180	0.0114	0.0181	...	0.0031
2a		0.0202		0.0027		0.0027	
2b	Method A	...	0.0202	0.0076	0.0103	...	0.0027
3a		0.0191		0.0036		0.0029	
3b	Method A	...	0.0191	0.0107	0.0143	...	0.0029
4a		0.0161		0.0017		0.0015	
4b	Method B	0.0043	0.0204	0.0100	0.0117	0.0031	0.0046
5a		0.0221		0.0034		0.0031	
5b	Method B	0.0039	0.0260	0.0133	0.0167	0.0010	0.0041
6a		0.0195		0.0014		0.0021	
6b	Method B	0.0058	0.0253	0.0153	0.0167	0.0015	0.0036
		Av.	0.0215		0.0146		0.0035
Direct assay		Av.	0.0223 ± 0.0023		0.0143 ± 0.0009		0.0039 ± 0.0004

a. Leached residue.
b. Leach precipitate.

The results obtained for the spectrographic analysis of fire assay beads in the leaching experiments are shown in Tables III, IV, and V.

DISCUSSION

The variables that were altered in an attempt to find the conditions which would result in maximum recovery of platinum metals can be seen in Table I. Attempts were made to correlate these variables with the results of analysis shown in Table II. No definite advantage was gained for the O or P samples by the use of large buttons, by reassaying, or by niter assays. In addition to these variables, large alterations were made in the amount of litharge used in the niter assays without obtaining improved recovery. Alterations in the amount of silica present in the flux, as well as oversize buttons and reassaying, failed to produce any outstanding advantage in the case of H samples. Results obtained by niter assay for H samples were very similar to those obtained by assaying the roasted samples. All the evidence above can be interpreted to mean that a wide variety of conditions give equally good recoveries of platinum, palladium, and rhodium from the samples tested.

One result for an O sample was deleted from Table III. It was considered to be due to the presence of a grain of platinum metals mineral. The presence of small, isolated grains of sperrylite could also account for the lack of precision with the ore samples. Scattered results are characteristic of analyses performed on samples in which the constituent sought is not intimately dispersed throughout the sample. Ideally, a few micrograms of platinum metals mineral must be distributed evenly in 30 grams of ore. This is not possible with any known methods of sampling and mixing. It is thus necessary to attach significance only to very large assays or to the averages of several small assays. Leaching and normal assay results agree closely when the ore is concentrated. Averages of platinum, palladium, and rhodium recovered from H samples by leaching (Table V) agree well with the normal assaying results.

In the case of the O and P samples the data in Tables III and IV may be interpreted only to indicate that leaching processes do not, in general, provide values higher than those obtained by normal fire assay. The results are not intended to define the precision which may be obtained by leaching processes. Undoubtedly more precise values could be obtained with O and P samples through the use of larger samples. However, the difficulties incident to the wet treatment of vary large amounts of ore encouraged the authors to limit their objective here to the question of the superiority of the leaching process.

The peculiar variations between the proportions of leachable and unleachable platinum metals are difficult to explain satisfactorily. Undoubtedly difference in grain size is an important factor.

ACKNOWLEDGMENT

Appreciation is also expressed to the Canadian Department of Agriculture, Science Service, for financial support and leave of absence given to I. Hoffman.

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RECEIVED for review December 9, 1955. Accepted March 5, 1956. Work supported by a grant from the National Research Council (Canada).

Determination of Polyphenol Oxidase Activity by Rotating Platinum Electrode

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Use of a polarized rotating platinum electrode enables polyphenol oxidase activity to be measured at various ascorbic acid concentrations, which is not possible with the commonly used chronometric method of Miller and Dawson. With this new method a continuous potentiometer record of oxygen consumption can be made.

THE catalytic activity of polyphenol oxidase, which is responsible for enzymatic darkening of fruits, is commonly described by a chronometric method that measures the time required for oxidation of a given amount of ascorbic acid (*3*, *9*) with catechol as substrate.

During studies in this laboratory it became necessary to determine the activity of polyphenol oxidase at various concentrations of ascorbic acid, which is impossible with the chronometric method. Because the rate of the reaction catalyzed by polyphenol oxidase is not constant, but falls off rapidly with time from reaction-inactivation (*8*), it is desirable to be able to measure the rate of reaction during the first few minutes. A polarized electrode for measuring the oxygen consumed in the reaction seemed to satisfy this requirement.

The first attempt with a polarized electrode was made with an alternating polarizing and depolarizing potential (*10*). Although this method was stable and accurate, 5 or 6 minutes were required for the cell to reach equilibrium after addition of the enzyme or substrate to initiate the reaction. This method was developed for photosynthesis studies (*2*) and would probably serve well in any determination where the first 5 minutes of the reaction are not so critical as with polyphenol oxidase.

However, a rotating polarized platinum electrode was found to reach equilibrium within 5 seconds. The use of a rotating platinum electrode to determine oxygen in solution is well known (*5–7*, *11*); its use in determining polyphenol oxidase activity by measuring the oxygen consumed in the reaction is described here.

EQUIPMENT

A schematic diagram of the equipment is shown in Figure 1. The reaction cell containing the rotating platinum electrode is polarized from the potentiometer with 0.800 volt. The current is measured by measuring the iR drop across R with a recording potentiometer. The variable resistance, R, is a standard decade resistance box which may be varied from 0 to 2000 ohms. The recording potentiometer has a range from 0 to 10 mv. and chart speeds of $1^1/_2$ and 6 inches per minute. Switch S is added to prevent erratic motions of the recorder pen when the cell is filled or emptied.

The cell, which contains 5 ml. of solution with the electrode inserted, is shown in Figure 2. The electrode is 2 mm. long and

0.25 mm. in diameter. Electrical contact is made with the electrode by means of the iron wire, *B*, dipping in the mercury pool. The 6-mm. glass shaft of the rotating platinum electrode, which is turned by a $^1/_{60}$-hp. induction motor at 1750 r.p.m., fits into the cell through a Teflon sleeve, *C*. The cell is emptied with an aspirator through the capillary tube, *D*, and is filled with a long-needled hypodermic syringe through capillary *E*. The excess solution and air bubbles exit through a hole, *F*, in the Teflon bearing. The cell is attached to a calomel cell through a salt bridge. The lower end of the salt bridge at *G* is stoppered with a sintered-glass plate and an agar plug saturated with potassium chloride. The whole cell is immersed in a 25.0° C. constant temperature water bath to the level shown at *H*.

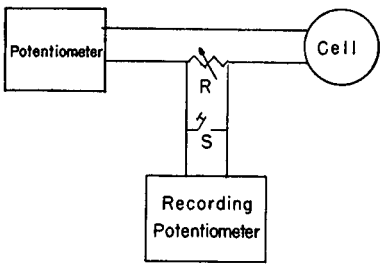

Figure 1. Schematic diagram of apparatus used to determine activity of polyphenol oxidase

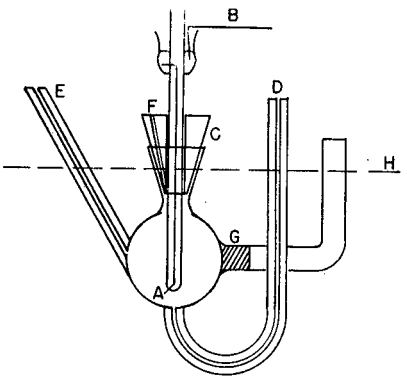

Figure 2. Cell used to determine polyphenol oxidase activity

A. Electrode
B. Electrical contact (iron wire)
C. Teflon sleeve
D. Capillary for emptying cell
E. Capillary for filling cell
F. Air and excess solution outlet
G. Lower end of salt bridge
H. Level of water bath

The current, measured by the recording potentiometer, is linear with the amount of oxygen present in the solution when the electrode is polarized with 0.800 volt. The intercept at 0% oxygen is the diffusion current for hydrogen ion in a solution which is 0.04*M* in disodium phosphate and 0.02*M* in citric acid. The solutions saturated with gases containing various percentages of oxygen were prepared by carefully adding a known volume of a solution saturated with air to a known volume of a solution saturated with nitrogen.

METHOD

The 5-ml. cell is filled with a solution 0.02*M* in citric acid, 0.04*M* in disodium phosphate, and 2 to 3*M* in ascorbic acid. The solution is saturated with air and contains varying amounts of substrate, depending upon which substrate is used. The pH of this solution is 5.5. The ascorbic acid is added to the reaction mixture to prevent *o*-quinone formation, because the polarized electrode will measure the *o*-quinone concentration in addition to the oxygen concentration. *o*-Quinone should not be allowed to accumulate in the cell because its polymerization products coat the electrode and cause the diffusion current to decrease. A coated electrode may be returned to approximately its original characteristics by a washing with dilute alkali or alcohol. Four or five volumes of reaction mixture are washed through the cell to eliminate any air bubbles. Resistance *R* is adjusted so that the recorder reads 9.5 mv. The enzyme, usually about 0.2 ml., is injected into the cell by means of a 0.25-ml. syringe. If the enzyme is not injected with sufficient force, the curves sometimes show a 1- or 2-second erratic period indicating a small mixing time. If the supply of enzyme is ample, this procedure can be reversed—i.e., the enzyme is added to the original reaction mixture and the reaction is initiated by the addition of substrate.

In Figure 3 is shown a tracing of recorder paper from an actual determination of enzyme activity. During the time *AB* the consumption of oxygen by the system without substrate is measured (blank). At point *B* the substrate catechol was added. Peak *C* is an initial time marker produced by momentarily closing switch *S* (Figure 1). The rest of the curve after *C* shows the uptake of oxygen by the system when catalyzed by polyphenol oxidase.

INTERPRETATION OF RESULTS

The plots in Figure 3 show that the oxygen-uptake curves are not linear with time but have a decided curvature. However, in the usual operation of the equipment, the curvature is not too great to determine the initial slope directly from the experimental curve by means of a prism or mirror. The most convenient range to measure is from 1 to 5 mv. per minute when the recorder speed is $1^1/_2$ inches per minute. From the solubility of oxygen in water saturated with air [5.78 ml. per liter (*4*)], the definition of a catecholase unit [10 cu. mm. of oxygen per minute (*8*)], the calibration curve, already described, and the fact that air is 6% less soluble in solutions 0.02*M* in citric acid and 0.04*M* in disodium phosphate than in water, one may calculate that 1 mv. per minute is equal to 0.40 catecholase unit. The chronometric method requires at least 50 catecholase units for an activity determination. This method requires considerably less enzyme, although 50 catecholase units itself is very little.

Figure 4 shows six individual determinations of activity by this method on solutions containing various volumes of a preparation of polyphenol oxidase from prunes.

Experimental. The preparation of prune enzyme parallels that

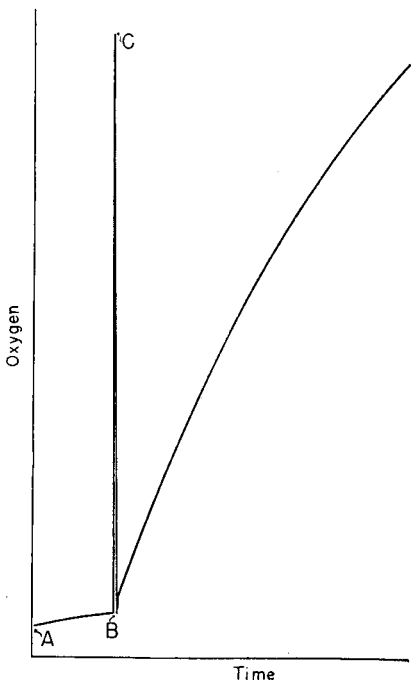

Figure 3. Direct tracing of actual recorder track showing oxygen consumption *vs.* time

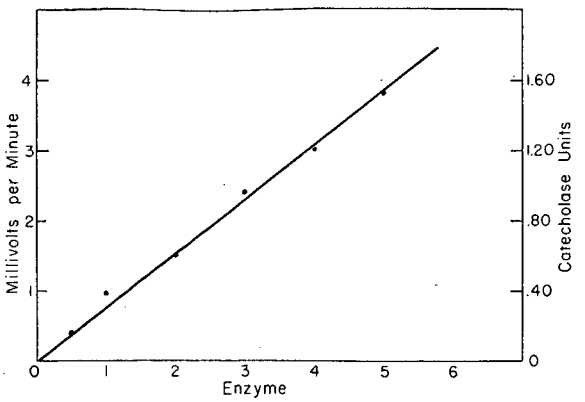

Figure 4. Rate of oxygen absorption measured by rotating platinum electrode at various amounts of polyphenol oxidase from prunes

Enzyme amounts are milliliters of enzyme per 100 ml. of reaction mixture; catecholase units would be 20 times that shown for undiluted enzyme

used to prepare potato enzyme by Baruah and Swain (*1*), except that 0.5% Pectinol (Rohm & Haas Co.) which contained no polyphenol oxidase activity was added to eliminate the pectin. The solubility of oxygen in the solution 0.02*M* in citric acid and 0.04*M* in disodium phosphate, relative to that in pure water, was determined with the rotating electrode by comparing a solution 0.02*M* in citric acid and 0.04*M* in disodium phosphate, saturated with air, with a solution of 50% of 0.04*M* citric acid and 0.08*M* disodium phosphate, saturated with nitrogen, and 50% of pure water saturated with air. After subtracting the diffusion current for a solution saturated with nitrogen, the diffusion current in the first solution was 6% less than twice that in the latter solution.

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RECEIVED for review October 8, 1955. Accepted March 29, 1956.

Preparation of Buffer Systems of Constant Ionic Strength

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Directions are given for the preparation of McIlvaine buffer systems of constant ionic strength, including a table of data.

THE importance of using adequately buffered solutions for the study of many types of chemical phenomena has long been recognized. The preparation of a large variety of buffer systems covering the usual range of pH is described in many reference and textbooks (*1*, *4–8*).

For certain purposes it is necessary to maintain the ionic strength of the solution relatively constant while varying the pH by varying the composition of one buffer system, as well as by using different buffer systems. For example, the polarographic half-wave potentials of certain types of organic compounds have been shown to be markedly dependent on the ionic strength of the test solution (*2*, *3*). The diffusion currents are also affected, although to a much lesser degree, and the slope of the polarographic wave is in some cases sensitive to ionic strength.

In the case of simple buffer systems such as those involving acetic acid–sodium acetate and ammonia–ammonium chloride, it is relatively simple to keep the ionic strength constant over the normal buffering range of the system corresponding to $pK_a \pm 1$. Bates (*1*) has described the preparation of a number of monobasic weak acid and monoacid weak base buffer systems of a definite ionic strength. However, in the case of more complicated buffer systems, such as those involving citrate and phosphate, it is much more difficult to keep the ionic strength constant. The usual directions for preparing these types of buffers result in large variation of ionic strength over the normal buffering range. Because the ionic strength depends upon the square of the charges on the ions present, the effect might be serious in

Table I. Preparation of Constant Ionic Strength McIlvaine Buffered Solutions

pH Desired at 25° C.	Composition, G./Liter Solution		Buffer System Ionic Strength, *M*	G. KCl Added per Liter of Solution to Produce Ionic Strength of	
	$Na_2HPO_4 \cdot 12H_2O$	$H_3C_6H_5O_7 \cdot H_2O$		1.0*M*	0.5*M*
2.2	1.43	20.6	0.0108	74.5	37.2
2.4	4.44	19.7	0.0245	72.7	35.4
2.6	7.80	18.7	0.0410	71.5	34.2
2.8	11.35	17.7	0.0592	70.2	32.9
3.0	14.7	16.7	0.0771	68.7	31.4
3.2	17.7	15.8	0.0934	67.6	30.3
3.4	20.4	15.0	0.112	66.2	28.9
3.6	21.5	14.2	0.128	64.9	27.6
3.8	25.4	13.6	0.142	64.0	26.7
4.0	27.6	12.9	0.157	62.8	25.5
4.2	29.7	12.3	0.173	61.7	24.4
4.4	31.6	11.7	0.190	60.4	23.1
4.6	33.4	11.2	0.210	58.9	21.6
4.8	35.3	10.7	0.232	57.2	19.9
5.0	36.9	10.2	0.256	55.5	18.2
5.2	38.4	9.75	0.278	53.8	16.5
5.4	40.0	9.29	0.302	52.1	14.8
5.6	41.5	8.72	0.321	50.6	13.3
5.8	43.3	8.32	0.336	49.5	12.2
6.0	45.2	7.74	0.344	48.9	11.6
6.2	47.5	7.12	0.358	47.9	10.6
6.4	49.6	6.47	0.371	46.9	9.62
6.6	52.1	5.72	0.385	45.8	8.50
6.8	55.4	4.79	0.392	44.5	7.23
7.0	58.9	3.70	0.427	42.7	5.44
7.2	62.3	2.74	0.457	40.4	3.10
7.4	65.0	1.91	0.488	38.2	0.488
7.6	67.2	1.35	0.516	36.0	..
7.8	68.6	0.893	0.540	34.3	..
8.0	69.6	0.589	0.559	32.9	..

going from a monovalent anion to a divalent anion over a pH range of 1 to 1.5 units.

In the attempt to minimize such changes in ionic strength, tables of buffer composition for the commonly used McIlvaine-type phosphate-citrate buffer system have been developed, which are helpful in the preparation of buffer solutions of uniform ionic strength throughout the normal buffering region of this system. Potassium chloride is added to the buffer compositions described in the literature, so as to keep the ionic strength constant at any desired level—e.g., 0.5*M*. These tables have been used in the authors' laboratories for several years in connection with studies of the polarographic behavior of organic compounds, and should be useful in other areas—e.g., investigation of reaction kinetics and spectrophotometric determination of pK values—in which ionic strength is a pertinent variable.

The essential data are given in Table I for McIlvaine buffers of constant ionic strengths of 0.5 and 1.0*M;* the amount of potassium chloride to be added for other ionic strength levels can be readily calculated on the basis of the ionic strength of the buffer system itself. Obviously, equivalent weights of other 1 to 1 electrolytes such as lithium chloride could be substituted for the weights of potassium chloride specified.

The specific ionic strength to which the buffer is brought will affect the actual pH of the solution to a slight extent. For this reason, the data in Table I are given only to the nearest 0.1 pH unit. The pH of the buffer solution as well as that of the final test solution should always be checked with a suitable pH meter.

ACKNOWLEDGMENT

The authors wish to thank the U. S. Atomic Energy Commission, which helped support the work described.

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RECEIVED for review January 16, 1956. Accepted March 6, 1956.

Techniques for Using Polytrifluorochloroethylene Plastic in the Chemistry Laboratory

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Apparatus of Fluorothene or Kel-F plastic, a polymer of trifluorochloroethylene, is very useful to the chemist in many cases where glass apparatus is unsatisfactory. A brief description of the useful properties of this plastic is given, along with some techniques of fabrication. A simplified technique for molding vessels from tubing is presented.

IN CASES where fabrication of laboratory apparatus with glass is undesirable because of special problems of flexibility, fragility, corrosion, surface activity, and thermal or electrical insulation, polytrifluorochloroethylene plastic may be used. This material is known by the trade names Fluorothene (Bakelite Co. registered trade-mark) and Kel-F (M. W. Kellogg Co. registered trade-mark). Often it is desirable to use Fluorothene (used in this text for all further reference to polytrifluorochloroethylene plastic) plastic instead of metals where high temperatures will not be used and transparency is important. Fluorothene plastic cannot be fabricated into useful laboratory equipment by ordinary means; however, various techniques successfully applied by the authors are set forth here.

PROPERTIES OF FLUOROTHENE

One of the most important properties of Fluorothene is its chemical inertness. As a polymer of monochlorotrifluoroethylene, its inertness is similar to that of Teflon, the completely fluorinated polymer. No effect has been observed after prolonged exposure to concentrated sulfuric, hydrofluoric, and hydrochloric acids, strong caustic, fuming nitric acid, aqua regia, and other vigorous oxidizing materials. Fluorothene is equally resistant to most organic solvents, but is slightly swelled and plasticized by highly halogenated materials and some aromatics (*3*). Other useful properties are high electrical resistance, thermal insulation, and stability. Dimensional stability is maintained over a temperature range from −200° to 190° C. Vessels of $^3/_4$-inch diameter or smaller, of approximately $^1/_{16}$-inch wall thickness, will withstand a high vacuum at 90° C. without collapsing. Fluorothene has much greater resistance to cold flow than Teflon. Although Fluorothene may deform slightly under applied pressure, it returns to its original shape when the pressure is released. It is relatively hard, having a Rockwell hardness of 111–115 (R- scale), and it can be machined into almost any desired form (*3*). Care must be taken to avoid excessive heating during machining. Fluorothene, when cooled slowly from high temperature, has a rather cloudy appearance, but attains transparency if quenched rapidly from 213° C. The material will decompose slightly above 270° to 300° C., depending upon its ZST value (*2*). [Zero strength time (ZST) is the time in seconds required to break a standard notched strip of heated polymer weighted with a small static load. This test, developed by the M. W. Kellogg Co. (*2*), provides a means of determining the ap-

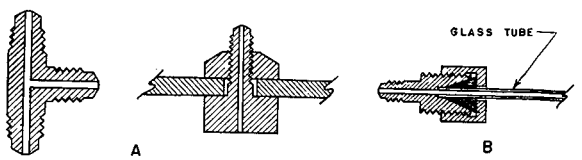

Figure 1. Fluorothene fittings for tubing connections

parent molecular weight of trifluorochloroethylene polymer and a basis for the grade designation of No. 270 and No. 300 for unplasticized material.]

Fluorothene decomposes slowly at 290° C. At this temperature possibly 15 to 30 minutes are required for serious decomposition; as the temperature is raised the rate of decomposition increases—that is, at 320° C. extensive decomposition may require only a minute or less. Teflon does not decompose until heated above 450° C. (*5*) and does not soften or melt below this temperature. The decomposition of Teflon proceeds at first with little visible change, there being no melting or darkening. Under vacuum a gas evolution may be observed at 389° C. (*4*), but when Teflon is heated in the air a temperature of 450° C. may be reached before decomposition can be detected by odor of the vapor or texture of the surface. The decomposition products are harmful and precautionary measures should be used.

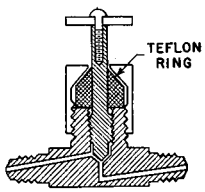

Figure 2. Fluorothene needle valve with Teflon packing ring and metal compression nut

Fluorothene plastic is exceptionally resistant to wetting by water. It is unaffected by high humidity, and water vapor transmission through unplasticized film above 0.002 inch in thickness could not be detected (*3*). Mercury wets the surface of Fluorothene much less than it wets glass. The surface of Teflon is more waxlike and its use as a packing gland, valve-stem guide, or valve seat permits ease of rotation when the rotating member is made of Fluorothene. The relative softness of Teflon makes it an excellent gasketing material in conjunction with Fluorothene or metallic surfaces.

FABRICATION

Fluorothene is available from the manufacturer in the form of extruded rod and tubing and molded disks and sheets (supplied by Plax Corp., Hartford, Conn., which recently sold its Fluorothene processing equipment to Westlake Plastics Co., Lenni Mills, Pa.).

Tubing Connections. The most convenient method of joining lines to vessels is by use of flared fittings similar to those used for copper tubing in refrigeration lines. Various types of machined Fluorothene fittings are shown in Figure 1, *A*.

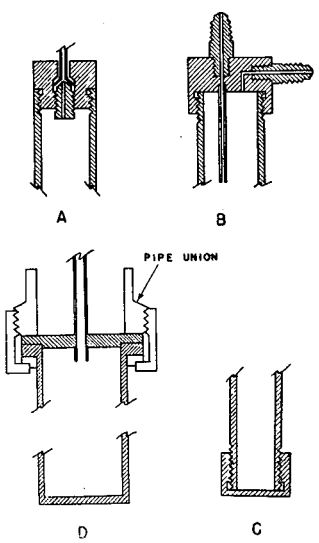

Figure 3. Fluorothene vessels constructed with compression seals

One-quarter-inch tubing was flared, using a conventional flaring tool. The tool was heated to approximately 150° C. and after the flare was formed the tool was not released until it had cooled to room temperature. When brass flare nuts are tightened on Fluorothene threads, care must be used to avoid stripping the threads. Glass tubing may be connected to plastic by means of a tapered Teflon packing ring sealed around the end of the glass tubing by a compression nut, as in Figure 1, *B*. A previous application of this principle employs a Teflon packing ring to connect glass tubing to metal (*1*).

Valves. The use of Fluorothene combined with Teflon in the construction of needle valves resulted in easy-turning, leakproof, and corrosion-free characteristics. A diagram of such a valve is shown in Figure 2. Metal parts were used to give rigidity, but in no case was metal permitted to come into contact with the material transported. Such valves are useful where greaseless stopcocks are desirable, in handling of organic solvents, and on vacuum lines for anhydrous hydrogen fluoride and other corrosive liquids.

Vessels. Vessels up to 1 inch in diameter were machined from extruded rod. Caps from solid stock of larger diameter were machined and threaded to the open end of the vessel. During the machining of Fluorothene plastic it was found that a slow feed and sharp tool provided the smoothest surface. At high speeds the plastic appeared to soften at the point of contact with the tool and a clean cut was not obtained. The slowest speed used to obtain a smooth surface was 50 r.p.m. and a feed of 0.185 inch per minute. In order to ensure a leak-tight joint a compression seal was made rather than sealing through the threaded joint itself as in conventional plumbing practice with iron pipe. Examples of compression seals are shown in Figure 3, *A*, *B*, and *C*. Lengths of tubing can likewise be sealed at both ends to construct leak-tight vessels. When the vessel is to be immersed in liquid nitrogen, thermal stresses may cause leakage at the threaded joints. This condition is much more serious when one of the joined members is made of metal. Fluorothene plastic will withstand liquid nitrogen temperatures without cracking and rapid warming does not appear to be harmful. Polyethylene, on the other hand, is entirely unsatisfactory in this respect.

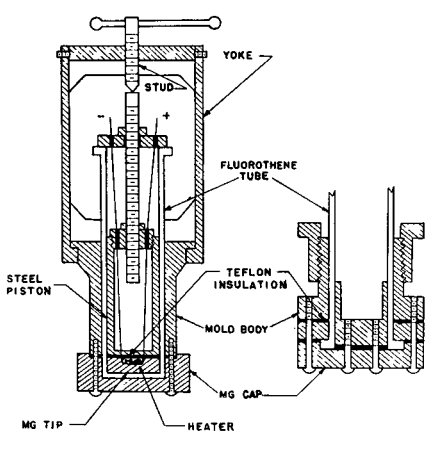

Figure 4. Plastic mold assembly

Vessels without threaded seals were constructed from tubing by a simplified molding technique. The open end of a tube could be closed by forcing it into a heated mold constructed as shown in Figure 4. By utilizing Teflon gaskets in the manner shown, it was possible to confine the heat to the very end of the plastic tube. In this way, pressure could be exerted on the upper cool end of the tube to force the tube into the mold body. Figure 4, *A*, shows the mold body for forming a flat bottom and Figure 4, *B*, the mold used for flanging the top of the vessel. The flanging operation should be done first, and best results are obtained with tubing $^3/_4$ to 2 inches in diameter.

In operation, an appropriate length of tubing was placed in the flanging mold (Figure 4, *B*). The magnesium (or aluminum) cap was slowly heated with a microburner until a temperature of 260° ± 5° C. was reached. (The plastic used corresponded to M. W. Kellogg Co.'s grade 270. For grade 300 a maximum temperature of 290° C. is desirable.) During the period from 225° to 260° C. pressure was maintained upon the tube by turning the stud at the top of the yoke (Figure 4, *A*). The end of the tube in contact with the heated cap was thus made to flow into the flanging mold. The 260° temperature was maintained for approximately a half hour, during which time the pressure was kept at a maximum. To prevent the upper wall of the tube from softening and collapsing, powdered dry ice was placed around the tube in the lower part of the yoke. The excellent insulating properties of the Teflon gaskets permitted the maintenance of a temperature gradient of about 200° between the heated cap and the upper part of the mold body. Finally, the entire assembly was rapidly quenched in ice water.

The mold (Figure 4, *A*) for forming the bottom of the vessel was used next. This was handled in much the same way as the flanging mold; however, it was necessary to employ an electrically heated tip on the steel piston. The heater consisted of a 0.5-ohm Nichrome wire embedded in Sauereisen cement and it was operated at 4 volts. When set up initially, the end of the plastic tube extended far enough below the piston tip to provide sufficient material for completely filling the mold when the position shown in the diagram had been reached. It was very important to clean the mold thoroughly after use and also to maintain the temperature below 270° C. Discoloration and decomposition resulted from excessive temperatures. (The darkening of Fluorothene is usually due to contamination by other organic materials or from plastic sources other than virgin material. The authors have felt that there may be an effect due to the magnesium surfaces of their mold.) It was equally important to keep the final temperature above 250° C. to prevent ridging and cracking of the plastic during its flow through the mold. Vessels so constructed were capped by a Fluorothene (or Monel) disk, which was held tightly in place by a slightly modified pipe union fitting (see Figure 3, *D*).

The vessels showed no sign of cracks, leaks, or deterioration after repeated immersions in liquid air under pressures in the range 0 to 2 atm. Some have been in continuous use for 3 years.

Capillaries. Capillaries of fine bore could not be prepared by drilling Fluorothene rod. However, it was possible to prepare capillaries of less than 0.1 mm. bore by the following method.

A 0.018-inch hole was drilled in a $^3/_8$-inch rod to a depth of 1 inch. The rod was then rotated slowly above a microflame until the plastic became transparent and pliable. A capillary was then drawn out in the same manner as with glass tubing. The plastic must be drawn very slowly and the center portion of smallest diameter should be permitted partially to harden before the drawing is completed. Otherwise, the softened rod may be broken before it has been stretched sufficiently. Small bulbs may be blown by first drilling and softening the plastic rod as for capillary preparation.

Sealing Electrode Leads. Electrode leads of platinum wire or other metal may be sealed into Fluorothene by first drilling a hole in the plastic a few thousandths of an inch undersize. By welding a wire of smaller diameter to the end of the electrode lead, one has a means of pulling the larger diameter wire into the plastic. The smaller diameter wire is slipped through the hole in the plastic, and by carefully heating the lead wire while pulling the wire is forced into the hole as the plastic softens. A wire above 0.050 inch in diameter may be successfully sealed into Fluorothene by employing a tapered Teflon packing ring, which is squeezed around the wire by a packing nut in a manner similar to the seal around the glass tubing as shown in Figure 1, *B*.

Test tubes and beakers of Fluorothene are available commercially for laboratory use. Furthermore, there is on the market a Kel-F grease which is a lower molecular weight polymer of trifluorochloroethylene. This can be used for lubrication of stopcocks and other surfaces exposed to corrosive reagents.

ACKNOWLEDGMENT

The authors wish to acknowledge the financial support of this work by the U. S. Atomic Energy Commission under Contract No. AT(11–1)–90, Project No. 3, Chicago Operations Office.

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RECEIVED for review November 18, 1955. Accepted February 29, 1956. Abstracted in part from the M. S. thesis of George Balog, Illinois Institute of Technology, February, 1954.

Simple Indicator Method for Determination of Aluminum

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A simple volumetric method was needed for control of aluminum concentration in certain solutions used in finishing aluminum. Such a method was developed, which is applicable in the presence of fluoride and affords accuracy and precision for dilute solutions.

THE method for aluminum developed by Bushey (*2*) involves the titration of an alkaline solution (Region *A*, Figure 1) to the point at which the free hydroxyl is neutralized (Region *B*, Figure 1), using pH measurements to determine the end point. Acid is then added through the region of precipitation of aluminum hydroxide to the point at which the precipitate is just redissolved (Region *C*, Figure 1). Potassium fluoride is added to precipitate cryolite in the acid solution, and the excess acid is titrated with standard base using phenolphthalein to the appearance of a pink color which remains for 15 seconds. The volume of hydrochloric used in the determination corresponds to the distance from *B* to *D* in Figure 1. Because this determination is not affected by the presence of fluorides, an attempt was made to simplify it without undue loss in accuracy or precision.

Preliminary experimental work with mixed indicators led to the conclusion that a determination based on the titration of an alkaline aluminate solution with standard acid to an end point in the pH range above 9 was feasible. The mixed indicator described by Kolthoff and Rosenblum (*3*), which has two color changes in the chosen pH range, was tested.

The first method attempted involved the use of two samples. Potassium fluoride solution was added to one sample to precipitate aluminum as cryolite. Both samples were then titrated to the green end point of the mixed indicator at pH 9.80. However, it was found that better precision and greater sensitivity were obtainable by using a single sample, titrating to the green color (pH 9.80), adding potassium fluoride solution at this point, and then titrating the hydroxide liberated from the aluminate ion by the fluoride to a yellow color at pH 9.35. The volume of

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Table I. Effect of Foreign Ions

(8.60 mg. of aluminum present)

Foreign Ion	Mg.	Treatment of Solution	Aluminum Found, Mg.	Aluminum Error, %
CO_3^{--}	12.49	None	8.71	1.28
	12.49	13.3 mg. $BaCl_2$ added	8.59	0.12
Cr^{+++}	5.44	None	6.32	14.9
	5.44	Boiled	8.23	4.32
	5.44	Boiled and filtered	8.59	0.12
F^-	18.9	None	8.57	0.35
	28.4	None	8.55	0.58
	37.8	None	8.50	1.16
Fe^{+++}	5.43	None	7.95	7.58
	2.71	None	8.44	1.85
	5.43	Boiled	8.57	0.35
	2.71	Boiled and filtered	8.60	0.00
NH_4^+	3.08	None	8.90	3.51
	6.16	None	9.29	7.90
	6.16	Boiled	8.59	0.20
SiO_3^{--}	3.53	None	8.36	2.80
	17.6	None	7.44	13.5
	35.3	None	5.72	33.4

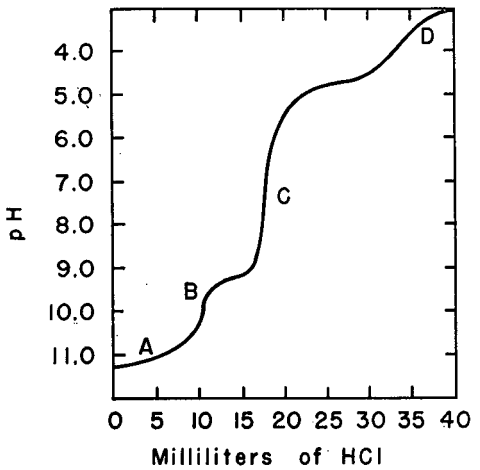

Figure 1. Titration curve for aluminum with hydrochloric acid

acid required for the titration after the addition of potassium fluoride was found to provide a direct measure of the aluminum content of the solution. The yellow end point at 9.35 was much sharper and easier to see than the green end point in solutions containing precipitated cryolite. The probable reactions which occur at various stages in this determination are shown in Equations 1 and 2.

$$AlO_2^- + OH^- + H^+ = AlO_2^- + H_2O \text{ (green end point)} \quad (1)$$

$$AlO_2^- + 2H_2O + 6F^- = AlF_6^{---} + 4OH^- \text{ (blue solution)} \quad (2)$$

These equations indicate that four hydroxyl ions are released per mole of aluminum. Experiments showed that an average of 3.6 moles of hydroxide are released per mole of aluminum. This loss of alkali is probably caused by a coprecipitation of hydroxide in the precipitated cryolite. The discrepancy may also be attributed in part to the fact that at pH 9.80, slightly more acid has been added than is required for the neutralization of excess alkalinity. This end point is near the pH required for precipitation of aluminum hydroxide and the solution may contain small amounts of precipitate, although a precipitate is not visible. Therefore, fewer than the four moles indicated in Equation 2 are released on the addition of fluoride. The change in pH during this determination is shown in Figure 2. The alkaline solution starting near *A* is titrated to the green end point, *B*, at which point the potassium fluoride solution is added. The hydroxyl ion released by the formation of the cryolite increases the pH to point *C*. This released hydroxyl ion is then neutralized to the yellow end point at *D*. Thus in the example given, which is for the standardization of acid against a known quantity of aluminum, the aluminum content is equivalent to 12.55 ml. of 0.092*N* hydrochloric acid.

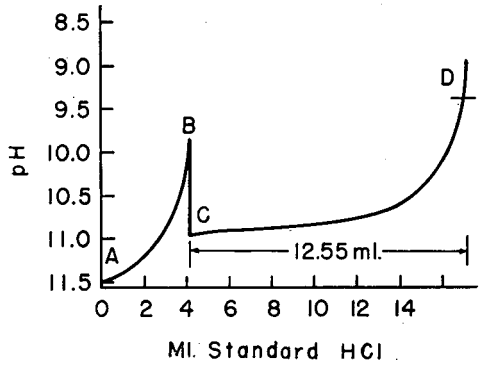

Figure 2. Standardization curve

0.603 mg. of aluminum; 1 ml. of hydrochloric acid = 0.6855 mg. of aluminum

A summary of the effect of foreign ions which interfere in this determination is listed in Table I. These include carbonates, silicates, ferric, chromic, and ammonium ions. Boiling the alkaline solution before titration removes ammonium ion; filtering removes the precipitated hydroxides of ferric and chromic ions. The low results obtained when iron and chromium are present may be due to coprecipitation of aluminum hydroxide with ferric or chromic hydroxide near the first end point (pH 9.80).

Barium chloride solution added after boiling, just before the initial titration is begun, serves to remove the carbonate. Thus, the most common interfering elements are easily removed.

EXPERIMENTAL

Reagents. Solution of approximately 0.1*N* hydrochloric acid, standardized as follows. High purity aluminum is dissolved in concentrated hydrochloric acid and an aliquot containing approximately 5 mg. is taken. The sample is diluted to 100 ml. and 10 drops of mixed indicator is added. The solution is then made strongly basic with sodium hydroxide pellets or carbonate-free sodium hydroxide solution. The color of the solution at this stage is deep violet. The strongly alkaline solution is then titrated to the green end point at pH 9.80 (point *B*, Figure 2) with dilute hydrochloric acid. A 20-ml. portion of 7% potassium fluoride is added to the reaction mixture, and the resulting solution is titrated to a yellow end point at pH 9.35 (point *D*, Figure 2) with the standard hydrochloric acid.

$$\text{Grams of aluminum per ml. of HCl} = \frac{\text{grams of Al used}}{\text{net ml. of HCl from } B \text{ to } D}$$

Reagent grade sodium hydroxide pellets or a carbonate-free solution of sodium hydroxide.

Anhydrous potassium fluoride, 7% solution, with the pH adjusted to 9.8 with sodium hydroxide. The pH adjustment can be made using the mixed indicator.

Mixed indicator, prepared by mixing 2 parts of 0.1% thymolphthalein and 1 part of 0.1% Alizarin Yellow in ethyl alcohol.

Procedure. No Interfering Substances Present. A sample of solution containing about 5 mg. of aluminum is diluted to 100 ml. with carbonate-free distilled water. Ten drops of mixed indicator is added, followed by solid sodium hydroxide or carbonate-free sodium hydroxide solution until the solution is strongly alkaline and has a strong violet color. The excess sodium hydroxide is titrated with hydrochloric acid until the solution is green (pH 9.80). The hydrochloric acid used for this titration need not be standardized. Then 20 ml. of 7% potassium fluoride is added to the solution. The resulting solution is then titrated with standardized hydrochloric acid to a yellow end point (pH 9.35.)

$$\text{Mg. of Al per sample} = \text{ml. of standard HCl} \times \text{mg. of Al per ml. of HCl}$$

In Presence of Interfering Substances. When ammonium, chromic, or ferric ion is present, the solution is made strongly

Table II. Influence of Aluminum Content on Accuracy and Precision

Aluminum, Mg. Present		Found
4.46		4.47, 4.46, 4.45, 4.45
	Av. dev.	0.22 part per hundred
	Std. dev.	0.01 mg.
	Coefficient of variation	0.22 part per hundred
	Av.	4.46 ± 0.07 mg. [99.9% confidence limits (*1*)]
8.92		8.96, 8.94, 8.87, 8.88
	Av. dev.	0.45 part per hundred
	Std. dev.	0.05 mg.
	Coefficient of variation	0.50 part per hundred
	Av.	8.91 ± 0.37 mg. (99.9% confidence limits)
22.30		21.78, 21.80, 22.78, 22.21
	Av. dev.	1.8 parts per hundred
	Std. dev.	0.47 mg.
	Coefficient of variation	2.1 parts per hundred
	Av.	22.14 ± 3.5 mg. (99.9% confidence limits)

basic as before and then boiled until a flocculent precipitate of chromic and/or ferric hydroxide is formed. The solution is filtered while still hot, and the residue is washed. The normal procedure is then followed with the combined filtrate and washings. When ammonium ion is the only interfering substance present, the basic solution need only be boiled to remove the ammonia before carrying out the normal procedure.

Care must be taken in handling the strongly alkaline solutions so that no carbon dioxide is introduced. In order to prevent reaction with glass and the formation of soluble silicates, the strongly basic solutions should not be boiled or stored in glass for excessive periods of time.

RESULTS

The method was developed to provide a simple determination for aluminum at low concentrations in certain solutions used in the surface treatment of aluminum alloys. Accordingly, an acidic fluoride solution used for the chemical brightening of aluminum, and in which control of the aluminum concentration is required, was used as a test solution. There is less than 1% error due to fluoride ion when the unknown sample contains less than 75 mg. of fluoride ion. Weighed amounts of foil (99.83% aluminum) were dissolved in the acidic fluoride solution and aliquots of the resulting solution, chosen to contain aluminum in amounts ranging from 4 to 22 mg., were analyzed by the mixed indicator method described above.

The results shown in Table II illustrate the effect of sample size on the accuracy and precision of the method. The average deviation, the standard deviation, and the coefficient of variation increase significantly with the aluminum content of the sample.

Bushey (*2*) has shown that the pH at which the excess alkalinity is neutralized increases as the concentration of aluminum in solution increases. For larger aluminum concentrations, therefore, the first color change at 9.80 in the present method is beyond the neutralization of the excess alkalinity.

However, for an aluminum content approximating that used in the standardization—i.e., 4.5 mg.—the accuracy and precision of the method are of the same order of magnitude as the method developed by Bushey. The simplicity of the present method permits the determination of aluminum without instrumentation by relatively untrained personnel. The major disadvantage of the method is the necessity for a preliminary determination to fix the sample size for optimum precision.

ACKNOWLEDGMENT

The authors wish to thank the Kaiser Aluminum and Chemical Corp. for permission to publish this paper.

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RECEIVED for review April 14, 1955. Accepted April 13, 1956

Methylmagnesium Chloride as Reagent for Determination of Reactive Hydrogen

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An improvement in the Zerewitinoff method for determining active hydrogen has been made by using methylmagnesium chloride in tetraethylene glycol dimethyl ether as the Grignard reagent. The new reagent has the advantage of low vapor pressure and excellent solubility for most organic compounds. Preparation of the reagent is described and results of active hydrogen determinations on several compounds are discussed.

THE so-called Zerewitinoff method for the determination of reactive hydrogen in organic compounds has been the subject of much investigation. A comprehensive review by Olleman (*3*) describes most of the literature concerning the method up to 1952.

After the work of Zerewitinoff (*9*, *10*), the majority of the literature concerned modification of the apparatus and procedure for the analytical technique. Few investigators used a Grignard reagent other than methylmagnesium iodide. Terent'ev, Shcherbakova, and Kremenskaya (*6*) used methylmagnesium chloride and reported that it lost titer on standing and was generally less reactive than the bromide or the iodide. Hückel and Wilip (*2*) reported the use of methylmagnesium bromide in isopentyl ether in the Zerewitinoff determination, as did Petrova and Perminova (*4*).

Because many organic compounds containing active hydrogen are insoluble in the solvent ordinarily used for the preparation of the Grignard reagent (pentyl ether), a number of secondary solvents have been required (*3*). Use of these additional solvents requires exacting purification and necessitates blank determinations for precise results. The choice of the secondary solvent may influence the amount of methane produced by the reaction, because of undesirable precipitation and other factors. A discussion of inconsistencies in the determination caused by different solvents has been reported by Wright (*8*).

Hill (*1*) found that many alkyl and aryl magnesium halides could be prepared in good yields by reaction in dialkyl ethers of glycols. The author has found that a preparation of methylmagnesium chloride in tetraethylene glycol dimethyl ether is an excellent reagent for the determination of active hydrogen by the Zerewitinoff method. Using an apparatus developed by Siggia (*5*), the reagent was tested with several alcohols and phenols using two of the common secondary solvents, pyridine and di-

oxane. Results were generally good, with many of the problems of solubility previously encountered having little effect on the determinations. The extremely low vapor pressure of tetraethylene glycol dimethyl ether was found to be an added advantage in preparation and subsequent use of the reagent.

PREPARATION OF METHYLMAGNESIUM CHLORIDE

In order to use a minimum of methyl chloride during preparation of the reagent, it was necessary to provide for maximum contact of the vapor with the magnesium. The problem was solved with a reaction vessel consisting of a glass column about 20 mm. in diameter and 150 mm. in length sealed to the bottom of a standard 250-ml. round-bottomed flask. The flask was fitted with a two-hole rubber stopper through which a drop tube made of 6-mm. glass tubing penetrated to the bottom of the column. The exit hole in the stopper permitted escape of excess methyl chloride and was fitted with a drying tube to prevent moisture from entering the system (Figure 1).

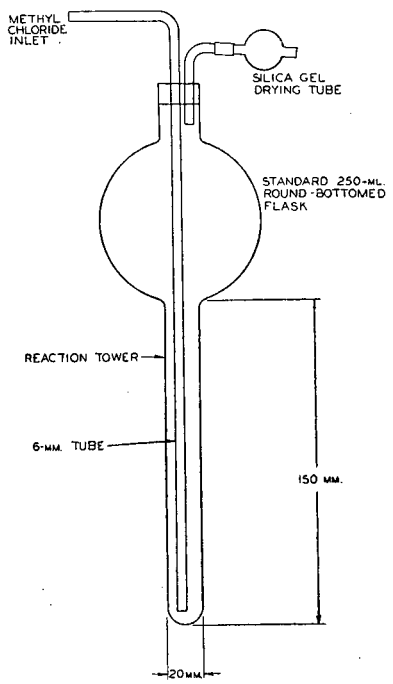

Figure 1. Apparatus for preparation of methylmagnesium chloride

Reagents and Procedure. In a typical preparation, about 7 grams of reagent grade magnesium turnings were made to react with excess methyl chloride in 200 ml. of tetraethylene glycol dimethyl ether. The commercially available material from Ansul Chemical Co. was found to be satisfactory. The final strength of the reagent is then about 1.4*M*. A higher proportion of Grignard reagent to solvent was found to cause some precipitation of magnesium salts. In contrast, a solution only 1*M* in methylmagnesium iodide in isopentyl ether causes continued objectionable precipitation on standing.

The magnesium turnings were introduced into the dry apparatus, filling the column around the drop tube. About 10 ml. of Grignard reagent from a previous preparation was added, along with enough tetraethylene glycol dimethyl ether to fill the column and cover the magnesium (about 30 ml.). Anhydrous methyl chloride vapor was then allowed to bubble slowly through the column. At the beginning of the reaction, the ether became cloudy from the reaction of the reagent with small amounts of impurities and water on the magnesium. When all of the magnesium became activated, the reaction became more rapid and exothermic, with subsequent clearing of the solvent. The remaining 170 ml. of ether was then added. The reaction was easily controlled by the rate of addition of methyl chloride. Because of the high boiling point of tetraethylene glycol dimethyl ether, it was not found necessary to cool the apparatus unless the reaction became too vigorous from a high rate of methyl chloride addition. In such cases, sufficient cooling was provided by directing a stream of air toward the hot parts of the column.

After completion of the reaction, the reagent was decanted through a filter of dry glass wool into a bottle fitted with a stopper and drying tube. The bottle was then heated on a steam bath for several hours to remove the excess methyl chloride in solution, after which it was fitted with a rubber serum cap through which portions of the reagent were subsequently drawn with a hypodermic syringe.

Activating Magnesium. After the Grignard reagent has been prepared, a small amount may be saved for initiating the reaction in future preparations. The magnesium for the first preparation was activated by an adaptation of the method of Underwood and Gale (*7*). A small quantity of magnesium turnings and several crystals of iodine were placed in a 10-ml. test tube and covered with 2 ml. of anhydrous ethyl ether. The reaction was allowed to proceed for 5 to 10 minutes, after which the ether was decanted and the test tube carefully heated to redness in a free flame. After cooling, about 5 ml. of tetraethylene glycol dimethyl ether, previously saturated with methyl chloride, was added to the test tube. The reaction started rapidly and proceeded until all of the methyl chloride was consumed. The magnesium and the Grignard reagent prepared in this manner were then used to initiate the reaction in the larger apparatus.

DETERMINATION OF ACTIVE HYDROGEN

Apparatus. The apparatus suggested by Siggia (*5*) was used for all active hydrogen determinations. The equipment appears to be the most simple and reliable of the many variations developed for this analysis. A detailed explanation of this apparatus and the manipulative procedure may be found in the original publication by Siggia. The only modification was the substitution of a 25-ml. buret for the original 7-ml. gas buret, permitting a slightly larger sample size for analysis.

Table I. Active Hydrogen Indicated by Alcohols and Phenols

Compound	Solvent	Sample, Mg.	Corrected Vol. of Methane, Cc.	Moles Active Hydrogen/Mole	
				Calcd.	Found
Methanol	Pyridine	17.3	13.3	1	1.10
		17.7	12.8		1.04
		17.5	12.8		1.05
Methanol	Dioxane	22.3	16.9	1	1.08
		22.6	16.4		1.04
		25.8	17.6		0.97
2-Butoxyethanol	Pyridine	62.4	12.4	1	1.04
		61.1	12.4		1.07
2-Butoxyethanol	Dioxane	29.3	6.3	1	1.13
		28.3	6.2		1.15
Ethylene glycol	Pyridine	25.2	17.9	2	1.97
		25.1	19.0		2.10
Ethylene glycol	Dioxane	18.3	12.6	2	1.91
		18.5	13.6		2.04
		18.9	14.2		2.09
Resorcinol	Pyridine	24.0	9.8	2	2.01
		23.6	9.6		2.00
		25.1	10.1		1.98
Resorcinol	Dioxane	25.8	10.6	2	2.02
		22.8	9.6		2.07
Hydroquinone	Pyridine	28.4	11.7	2	2.03
		27.8	12.1		2.13
Pyrogallol	Pyridine	26.1	13.6	3	2.92
		26.0	14.0		3.02

Procedure. About 3 ml. of the Grignard reagent was transferred by means of a dry hypodermic syringe from the reagent bottle to the serum-capped reaction flask on the apparatus. The reagent was then stirred and heated in a steam bath for 10 minutes, after which it was allowed to cool with stirring for 20 minutes. This blank determination on the reagent permitted reaction with small amounts of water that may have remained in the apparatus. After sweeping with dry nitrogen, the gas buret was leveled and prepared for addition of the sample. The sample for determination was weighed in a 2-ml. hypodermic syringe and introduced through the serum cap on the reaction flask in the same manner as the reagent. After stirring and initial leveling of the mercury in the gas buret, the reaction flask was heated for 10 minutes and cooled to room temperature with stirring as be-

fore. The observed volume of methane was then corrected for temperature and volume of sample added and calculated as moles of active hydrogen in the sample.

RESULTS

Tetraethylene glycol dimethyl ether is an excellent solvent for many organic compounds containing active hydrogen. However, in order to compare the results obtained using the methylmagnesium chloride reagent with previous data (*8*), pyridine and dioxane were chosen as solvents for several compounds containing active hydrogen, as shown in Table I. Known samples were prepared by weighing appropriate amounts of the glycols and phenols into small, serum-capped flasks and diluting with the solvent. There was very little precipitation of the reaction products in pyridine. Dioxane gave no precipitate with the alcohols of low molecular weight, although there was some precipitate with the phenols. Hydroquinone and pyrogallol gave erratic results accompanied by the formation of large amounts of precipitate when dioxane was used as the solvent. It was felt that at least part of this difficulty was due to the fact that thorough agitation of this precipitate was not achieved, and as a result the reaction was incomplete.

In the author's laboratory, the reagent has been widely used in conjunction with the Karl Fischer reagent for the determination of both water and active hydrogen compounds in several ethers of glycols. The water found by the Karl Fischer reagent may be calculated as active hydrogen and subtracted from the total determined with the Grignard reagent, the difference then being the active hydrogen other than that due to water.

In contrast to the usual properties of chloride Grignard reagents in ethereal solvents, the reagent prepared in tetraethylene glycol dimethyl ether showed little tendency to precipitate magnesium salts when stored for at least 1 month. No loss in titer was observed at the end of such storage. However, during storage the reagent must be kept free from moisture in a container such as that described so that it can be withdrawn without contamination.

The reagent has not as yet been applied to the determination of compounds which react with Grignard reagent by addition or coupling. It is expected to be of further value for this type of analysis.

ACKNOWLEDGMENT

The author wishes to thank the Ansul Chemical Co. for permission to publish this work and Philip Ehman for his valuable help in preparing the manuscript.

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RECEIVED for review December 12, 1955. Accepted February 27, 1956.

Improved Spot Test for Boron and a Quantitative Estimation of Boron in Very Dilute Solutions

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The standard spot tests for boron have been evaluated experimentally with particular attention to their reproducibility and to the substances that interfere with the tests. Most of the standard spot tests for boron were unsatisfactory for practical use with very dilute solutions. A superior spot test for boron, using sorbitol, combines ease of procedure, reproducibility, and high sensitivity. Based on the mechanism of this spot test, and using a series of polyhydric compounds, a method for the quantitative estimation has been developed.

BECAUSE of the recent developments in the use of boron in the atomic energy program, and because of the critical level of boron content in agricultural soils, a rapid and sensitive method for the detection of boron in very dilute solutions (less than 1 p.p.m.) is most desirable. Several relatively sensitive spot tests for boron are described in the literature. In all these tests, however, the limit of concentration is too high to permit a reliable detection of boron in very dilute solutions.

In an attempt to extend the limit of concentration of some of these spot tests so that the tests could be applied to very dilute solutions, a survey of the more applicable of the available tests has been made.

A study of the quinalizarin test (*3*, *4*) revealed that when the volume of the test solution is increased to 1 ml. (instead of the recommended few drops) the quantity of base and/or salts present, as well as the degree of drying, becomes critical. The degree of drying is easily controlled, but the quantity of base present varies as the volume of solution, at constant pH. The presence of an excess of either base or sodium salts causes a red coloration which masks the blue of the boron complex.

Similarly it was found that when chromotrop-2B (*3*) is used as the color-developing reagent, an orange color, which obscures the test for boron, is developed in the presence of even relatively low concentrations of sodium salts.

Although no interfering color is developed by sodium salts in the curcurmin (*1*) test for boron, the sensitivity of the test is so decreased by the presence of salts that the test is of little value in working with very dilute solutions.

The spot test with the lowest limit of identification described in the literature is the mannitol test (*2*, *5*). No common substances interfere. It seemed logical, therefore, to begin the search for a superior test with an investigation of this reaction. Mellon and Morris (*7*) determined the titration curves of boric

acid in the presence of many polyhydric compounds. From these curves it is apparent that the three isomers mannitol, ducitol, and sorbitol yield boron complexes of different degrees of acidity. In two cases they found that the boron-polyhydric complex was a stronger acid when in the presence of a saturated solution of sodium chloride. This effect of sodium salts increasing the acidity of the complex has also been observed by Tanino (*8*). Krantz (*6*), too, has demonstrated the variation in the acidity of the boron-polyhydric complex while using polyhydric compounds of a similar nature.

The work of Mellon and Morris (*7*) indicated the possibility of developing a number of spot tests for boron using various polyhydric compounds, each test having a different sensitivity. It should be possible to increase the sensitivity of the test by the addition of sodium chloride. Thus, mannitol, sorbitol, glycerol, glucose, invert sugar, and *cis*-2,3-butanediol should all give tests for boron, but of varying sensitivity.

Table I. Sensitivity of Boron Spot Tests

Compound	Sensitivity, γ/ml. No sodium chloride	With 1 ml. sodium chloride	With 2 drops sodium chloride solution
cis-2,3-Butanediol	2	Reversal	2
Glucose	5	Reversal	1
Invert sugar	0.5	Reversal	0.5
Glycerol	0.5	Reversal	0.04
Mannitol	0.02	0.01	0.01
Sorbitol	0.01	Reversal	0.003

REAGENTS

All solutions and reagents were prepared in porcelain or fused silica ware and stored in polyethylene bottles.

Mannitol. Matheson Co. mannitol.

Sorbitol. Atlas Powder Co. crystalline D-sorbitol.

Glycerol. One to 1 solution of glycerol in water, neutralized so as to give no blank in the test procedure.

Invert sugar. A 20% solution of sucrose, hydrolyzed with hydrochloric acid and neutralized so as to give no blank in the test procedure.

Glucose. A 20% solution of glucose, decolorized with activated carbon, and neutralized so as to give no blank with the test procedure.

cis-2,3-Butanediol. Neutralized so as to give no color change with a blank test.

Sodium chloride solution. Twenty-five grams of Baker's analyzed sodium chloride in 125 ml. of water.

Hydrochloric acid, 0.02 and 0.002*N*.

Sodium hydroxide, 0.02*N*.

Standard boron solution. Stock solution of 100 γ boron per ml. prepared from Baker's analyzed boric acid. Further standards of 10, 1.0, 0.10, and 0.010 γ of boron per ml. prepared by dilution.

A modification of the mannitol method of Hahn (*5*) was chosen as the most applicable to this study. The method is based upon the colorimetric detection of the increased acidity of a boric acid solution upon the addition of a polyhydric compound.

Modified Procedure. Place 1 ml. of the test solution in a size 1 Coors porcelain crucible. Add 1 drop of phenolphthalein and 2 drops of the sodium chloride solution. Make alkaline to phenolphthalein with 0.02*N* sodium hydroxide. Evaporate to dryness on a steam bath. Take up the residue in 1 ml. of water. Barely discharge the phenolphthalein color with 0.02*N* hydrochloric acid. Add 2 drops of bromthymol blue and adjust to a greenish yellow with 0.002*N* hydrochloric acid. Place 3 drops of this solution in each of two adjacent depressions of a white porcelain spot plate. To one spot add a little solid or 1 drop of solution of the polyhydric compound. A shift in color toward the yellow indicates the presence of boron.

Two variations of the above procedure were tried with each of the polyhydric compounds. In the first variation no sodium chloride was added to the solution. In the second variation no sodium chloride was added to the solution prior to evaporation, but the residue was taken up in 1 ml. of the sodium chloride solution.

In several instances, it was noted that when the alkaline residue was taken up in the sodium chloride solution, the results of the test were opposite to those which were expected. That is, when the polyhydric compound was added to the solution, the color shifted toward the blue or basic side instead of toward the yellow or acid side. No explanation of this phenomenon is offered here.

EXPERIMENTAL

Mannitol. It was found that by taking up the alkaline residue from evaporation with 1 ml. of the sodium chloride solution a sensitivity of 0.01 γ of boron was obtained, which is double the sensitivity of the Hahn test. The same results were obtained by adding 2 drops of the sodium chloride solution before evaporation.

Sorbitol. In the absence of sodium chloride in the test solution 0.01 γ of boron could be detected. When the alkaline residue was taken up in the sodium chloride solution, a reversal of the test was obtained. However, when 2 drops of the sodium chloride were used, the sensitivity of the test was increased so that 0.003 γ of boron could be detected.

Glycerol. Using glycerol alone, a sensitivity of 0.5 γ per ml. of boron was obtained. As with sorbitol, a reversal of the test was obtained when the residue from the evaporation was taken up in 1 ml. of the sodium chloride solution. Again, the sensitivity was increased when 2 drops of the sodium chloride solution were added before evaporation, so that 0.04 γ per ml. of boron could be detected.

Glucose. Using glucose alone in the test, a sensitivity of 5 γ per ml. was obtained.

When the residue from evaporation was taken up in 1 ml. of the sodium chloride solution, a reversal of the test was obtained. However, when 2 drops of the sodium chloride were added to the test solution before evaporation, 1 γ per ml. could be detected.

Invert Sugar. Using invert sugar alone, a sensitivity of 0.5 γ per ml. of boron was obtained. No increased sensitivity was obtained when 2 drops of the sodium chloride solution were used, but a reversal of the test was obtained when the residue from evaporation was taken up in 1 ml. of the sodium chloride solution.

***cis*-2,3-Butanediol.** Using butanediol alone, a sensitivity of 2 γ per ml. of boron was obtained. An extremely strong reversal of the test was obtained when the residue from evaporation was taken up in 1 ml. of the sodium chloride solution, and there was no increased sensitivity when 2 drops of the sodium chloride solution were added before evaporation.

Table I summarizes the sensitivities of the polyhydric compounds when the three modifications of the test procedure were used.

The use of the term "limit of identification" is somewhat misleading, as only approximately 20% of the test solution is actually used in the color development. However, for the purpose of simplicity the sensitivity of the case is designated as the number of micrograms of boron that may be detected in 1 ml. of the test solution.

Table II. Interfering Ions

Interfering Ion	Sorbitol, γ	Mannitol, γ	Invert Sugar, γ	Glucose	*cis*-2,3-Butanediol, γ	Glycerol, γ
HSO_4^{--}	...	...	..	..	..	50
NO_2^-	...	...	..	..	50	..
Ca^{++}	...	...	..	..	50	..
Hg^{++}	...	...	..	..	50	..
MoO_4^{--}	12.5	25	..	..	..	50
VO_3^-	50	50	..	..	..	..
WO_4^-	6.25	6.25	50	..	..	50
Fe^{+++}	50	...	..	..	..	..

Interfering Ions. The test for boron was performed at maximum sensitivity with each of the compounds mentioned above in the presence of diverse foreign ions. In each case, the original amount of foreign ion present was 100 γ. If no decrease in the sensitivity of the test was observed with this quantity of the

ion, the ion was considered as noninterfering. If the sensitivity of the test was reduced, the quantity of the interfering ion was successively reduced until the maximum sensitivity was obtained. The ions that do not interfere with any of the tests are: CO_3^{--}, SO_4^{--}, NO_3^-, Sr^{++}, Pb^{++}, PO_4^{---}, $C_2H_3O_2^-$, Bi^{+++}, Mn^{++}, BrO_3^-, Cd^{++}, Mg^{++}, Al^{+++}, Zn^{++}, Ti^{++++}, Sn^{++}, Ag^+, Sb^{+++}, Co^{++}, Cu^{++}, Ni^{++}, Cr^{+++}, $H_2PO_4^-$, and Ba^{++}. Table II lists the interfering ions for each test, with the tolerance of each compound for that ion. None of the ions above, or in Table II, gave a positive test in the absence of boron.

Standard methods for the quantitative determination of boron in very dilute solution are tedious, time-consuming, and not highly accurate. Therefore, it seemed desirable to extend this work to include an estimation of the boron concentration of the test solutions. The varying sensitivities of the spot tests described serve as a basis for the development of a rapid simple method for the quantitative estimation of boron concentration.

The method for the estimation consists simply of repeating the spot test procedure on the same solution but with different polyhydric compounds. After the pH is adjusted with bromothymol blue, the solution is tested with each of the compounds of Table I, in order of decreasing sensitivity, until no test is obtained. The concentration then lies between the sensitivity of the last compound giving a positive test, and the sensitivity of the first compound giving a negative test.

This method of quantitative estimation does not give the exact concentration of boron in a sample, but rather the range into which the concentration falls.

In order to check the application of these polyhydric compound spot tests to the quantitative estimation of boron, several solutions of known concentration were prepared. The boron concentration was then determined as previously described with the results shown in Table III.

Table III. Estimation of Boron

Boron Present, γ/Ml.	Boron Range Found, γ/Ml.
0.2	0.04 to 0.5
0.25	0.04 to 0.5
0.05	0.04 to 0.5
0.05	0.04 to 0.5
0.0025	0.003 to 0.01

ACKNOWLEDGMENT

The authors gratefully acknowledge the assistance of A. F. Isbell of the A & M College of Texas, who prepared the *cis*-2,3-butanediol.

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RECEIVED for review December 17, 1955. Accepted March 19, 1956.

Fire Assay for Platinum

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The efficiency of the fire assay for platinum has never been determined. These researches were undertaken to gain information concerning the influence of flux and ore composition upon the accuracy of extraction of platinum by lead, and to determine the degree to which platinum is lost to the cupel. It was found that a wide variety of types of fluxes provide acceptable recovery of platinum. For ores containing high proportions of nickel, losses may occur to the slag. Fire assays of materials containing finely divided platinum or its salts may result in migration of platinum to the pot wall. Cupellation losses were negligible. The results will find applications in the fire assay for platinum ores and concentrates, and in the cupellation of lead buttons. The effect of nickel is important because it is commonly present in platinum metal ores.

THE established methods for the determination of platinum metals in ores and concentrates utilize fire assay procedures for the preliminary separation from the gangue. The success experienced in fire assaying for gold and silver, rather than experimental data, has formed the basis for accepting these methods.

Platinum metals, with the possible exception of platinum itself, are more disposed to oxidize than gold or silver and the possibility that these oxides would react with the basic and acidic oxides present in dry reaction mixtures led to the conception that these metals might be incompletely recovered. A loss might likewise be anticipated in cupellation with platinum metals dissolving in the molten litharge. Results from systematic investigations in this laboratory over a period of years have in some cases supported these concepts.

Variable losses for all types of slags were noted for ruthenium, and high losses occurred to the cupels on cupellation (*10*). Losses were lower but not constant for given slag compositions and fusion temperatures in the case of rhodium (*2*). Losses of 1 to 6% were encountered in the fire assay for osmium, but nearly complete volatilization during partial or full cupellation made wet treatment of the complete button necessary (*1*). Reassaying of the slags was required for complete collection of iridium, and significant losses occurred with basic slags, while base metals caused up to 50% losses. The cupellation of lead-iridium buttons resulted in serious mechanical loss of iridium (*4*). In the case of palladium, collection was good except where the slag contained considerable nickel. Cupellation losses were not significant (*7*).

During an initial evaluation of the probable difficulties to be encountered in the present investigation, the guess was made that chemical loss to slags would be small because of the high oxygen dissociation pressures of the known platinum oxides. However, the possibility of compound formation, other than with oxide, with some flux constituents could not be excluded.

In the present paper the authors present data obtained from an examination of the collection of platinum by lead from various fluxes, and from the cupellation of lead buttons containing platinum, and show the degree of interference caused by the base

Table I. Composition of Fluxes

Flux No.	Type of Flux	Silica Sand, %	Borax Glass, %	Calcium Oxide, %	Soda Ash, %	Litharge, %	Potassium Nitrate, %	Weight Taken per Assay, Grams
1	Glazing flux	20.0	7.5	5.0	27.5	40.0	..	35
2	Very acid (medium viscosity)	25.7	5.4	6.7	..	62.6	..	80
3	Very acid (low viscosity)	14.3	30.0	..	27.2	28.5	..	70
4	Basic	11.5	1.8	2.7	20.3	63.7	..	100
5	Neutral (without silica)	..	26.8	6.1	42.7	24.4	..	80
6	Neutral	15.4	7.7	6.4	44.9	25.6	..	78
7	Oxidizing (niter assay)	4.0	2.6	..	16.9	58.8	17.7	133[a]
8	Base metals flux	14.9	..	..	7.5	77.6	..	200

[a] Sulfur (6.2 grams) and litharge (28 grams) were mixed with this flux before salting in a cellophane liner.

metals most often encountered. The losses caused by preliminary fusion of the different fluxes with platinum in order to encourage the formation of resistant compounds are discussed. An attempt has also been made to throw some light on the reason for the small losses of platinum metals which occur during the assay of ores and which are not eliminated by reassaying.

APPARATUS AND REAGENTS

Apparatus. The fire treatments were made in a pyrometrically controlled Williams and Wilson 15 KVA Globar-type assay furnace.

Transmittance measurements were made with a Lumetron 402 EF colorimeter using a Photovolt narrow-band filter centered at 390 mμ.

Reagents. Platinum Solution. A stock solution was made from spectrographically pure powdered platinum sponge obtained from Johnson, Matthey, and Mallory, Ltd., Toronto, Ont. A 2.008-gram portion was dissolved in aqua regia and the solution was evaporated to dryness on a steam bath. Aqua regia was added and the solution was evaporated again; this procedure was repeated a third time with aqua regia, hydrochloric acid, and water. The residue was taken up in dilute hydrochloric acid and the solution was filtered. The volume was made up to 4 liters of solution containing 40 ml. of concentrated hydrochloric acid. This solution was standardized by benzenethiol (*5*), and the results from three successive 9.98-ml. portions were: 4.97, 4.99, and 5.01 mg.

Benzenethiol Reagent. A 10% by volume alcoholic solution of benzenethiol (Eastman Kodak Co., Rochester, N. Y.) was prepared freshly before use.

Tin(II) Chloride Solution. Tin(II) chloride solution, 1.0*M* in tin(II) chloride and 3.5*M* in hydrochloric acid, was prepared from Baker and Adamson reagent grade tin(II) chloride dihydrate. The salt was brought into solution by heating with concentrated hydrochloric acid. After being filtered through a sintered-glass funnel, the solution was diluted to volume, and stored under a layer of xylene to protect against atmospheric oxidation.

Sodium Chloride Solution. One hundred twenty-five grams of British Drug House reagent grade sodium chloride were dissolved in 500 ml. of solution.

EXPERIMENTAL

Composition of Fluxes. The composition and quantities of the fluxes used are recorded in Table I. Some are identical with those used in this laboratory for the investigation of other platinum metals (*1*, *7*).

Method of Salting Fluxes. An 8-inch square of cellophane sheet was used as a lining for either a 15- or 20-gram assay crucible. The charge was made by rolling on a cellophane square 80% of the flux required for the assay together with sufficient litharge and flour to produce a 25-gram lead button. A known amount of standard platinum solution was added to a depression in the center of the charge and the pot and contents were dried overnight in an oven at 105° C. The remaining 20% of the flux was added as a cover after the ends of cellophane square were folded over and pushed down the sides of the pot with a spatula. One gram of flour was deducted from the charge to allow for the litharge reduced by the cellophane.

Fire Assay Procedure. The pots were placed in the furnace at 975° C. and the temperature was raised at the maximum rate to 1150° or 1200° C. The fusion mixtures were poured into conical iron molds and, when cool, the slags were broken away from the lead buttons. All the slag from a single assay was ground to pass a U. S. standard sieve No. 45, and reassayed with added litharge and flour in the original pot.

Prefusion Experiments. Eighty per cent of the flux was placed in a pot glazed with flux 1 without the cellophane liner, flour, or added litharge. Silver nitrate solution to give a 75-mg. silver bead on cupellation was added along with the standard platinum solution and the pot and contents were dried as before. Fusion and grinding were done as described above, and assays were made in the original pots after mixing with flour and extra litharge. Results obtained using this procedure showed very poor recoveries for basic and neutral fluxes. These might be interpreted to mean that large amounts of platinum had gone into the slag in a form which resisted collection. This led to an extensive examination of the ground slag for the presence of platinum but negative results were obtained by spectrographic analysis, fusion methods, and treatment with hydrofluoric acid.

Attention was then directed to the possibility that in a preliminary fusion some platinum might migrate to the pot walls and remain there when the fusion mixture was poured. It was definitely shown that pot wall losses occur by an experiment on the pot in which flux 2 was fused with 5 mg. of platinum without collection by lead. A very large recovery of platinum was obtained from the walls by assaying with flux 6 containing added litharge and flour. As a result of this finding further prefusion experiments were undertaken. The original fusion pot was examined separately for pot wall loss with flux 6 and the prefused slag was assayed and reassayed several times in a new pot. In addition, the original pot and slag from the first fusion were reassayed by a second fusion of the ground slag with extra litharge and flour. Flux 6 was also used for a final check on the second pot. Results obtained are shown in Table II.

Table II. Distribution of Platinum in Fire Assay after Prefusion

(Including assay of pot. 1000 γ of platinum taken. Range of weights of lead buttons, 20 to 34 grams)

	Amount of Platinum, γ				
Flux	Acid	Acid	Basic	Neutral	Neutral
Flux No.	2	3	4	5	6
Assay of pot wall	745	530	545	17	2
Reassay of pot wall	27	19	25	3	1
Cupel assay	5	4	2	0	0
1st slag	200	435	140	110	890
1st cupel assay	0	0	0	0	11
2nd slag	16	6	0	0	0
3rd slag	3	2	0	0	0
4th slag	2	1	0	0	0
Final pot wall assay	0	2	0	0	0
Total recovered	998	999	712	110	904
Platinum unrecovered	2	1	288	890	96

COLORIMETRIC DETERMINATION OF PLATINUM IN LEAD BUTTONS

A method was needed for the determination of platinum in amounts up to 1 mg., which could be applied in various stages of the investigation. From a review of the literature it was considered that thiosemicarbazide offered considerable promise as a reagent. It gave a good blue color with platinum, but as the data published by De Trecco (*6*) were incomplete, the authors undertook a more detailed study. A maximum absorbance was found at 585 mμ and a pH of 6.4 was found to be optimum for the final solution. Color instability and failure to obtain good reproducibility were serious problems. When these difficulties were further complicated by interference from small quantities of base metals present after assaying, it was decided to turn to tin(II) chloride as the reagent.

Table III. Distribution of Platinum in Fire Assay

(Samples of 1 mg. and under)

Flux	Flux No.	Button Weights, Grams: 1st	Parting acid assay	Reassay of parting acid	2nd	Platinum Found, γ: 1st button	Parting acid assay	Reassay of parting acid	Total in 1st button	2nd button	Total Pt Recovered, γ	Pt Taken, γ	Difference, γ
Acid		24	26	28	25	65	28	7	100	0	100	100	0
		24	26	21	28	140	55	5	200	33	233	250	−17
		22	28	17	29	375	77	23	475	18	493	500	− 7
		24	27	23	27	840	103	3	946	24	970	1000	−30
Acid	3	32	..		29	70	5		75[a]	0	75[a]	100	−25
		30	..		30	220	5		225	4	229	250	−21
		35	..		29	485	10		495	2	497	500	− 3
		29	..		30	965	35		1000	3	1003	1000	+ 3
Basic	4	34	22		25	90	0		90	5	95	100	− 5
		32	24		26	220	0		220	20	240	250	−10
		33	23		25	485	8		493	8	501	500	+ 1
		33	23		23	980	19		999	5	1004	1000	+ 4
Neutral	5	31	..		27	65	20		85	0	85	100	−15
		32	..		26	180	35		215	0	215	250	−35
		33	..		26	415	40		455	6	461	500	−39
		32	..		28	935	65		1000	5	1005	1000	+ 5
Neutral	6	36	..		25	95	8		103	1	104	100	+ 4
		36	..		23	230	7		237	0	237	250	−13
		36	..		23	480	8		488	0	488	500	−12
		37	..		22	945	27		972	1	973	1000	−27
Oxidizing	7	26	29		31	75	13		88	0	88	100	−12
		27	30		30	215	17		232	0	232	250	−18
		27	28		31	465	34		499	1	500	500	0
		27	28		31	925	40		965	1	966	1000	−34

[a] These numbers omitted from averages shown in Table VI.

Various factors which affect the accuracy of the tin(II) chloride method are given in detail by Ayres and Meyer (*3*). Small amounts of base metals (chiefly lead) present after aqua regia treatment were found by the present authors to be without adverse effect. Sodium chloride solution was added before the aqua regia treatment to avoid the possibility of platinum baking out on the walls of the beaker when the solution was evaporated to dryness.

Nitric Acid Parting. Lead buttons containing platinum in amounts up to 1 mg. were analyzed colorimetrically and for those with 5-mg. quantities gravimetric methods were used. The button was placed in 150 ml. of 1 to 4 nitric acid and allowed to part overnight on the steam bath. The residue was recovered on a Whatman No. 42, 9-cm. filter paper and washed repeatedly with hot water. The paper, after being folded, was placed in a 30-ml. beaker and ignited overnight in an electric oven at 400° C. Sodium chloride solution (0.5 ml.) and aqua regia (2.0 ml.) were added and the solution was evaporated to dryness on a steam bath. The residue was taken up with several drops of aqua regia and the solution was evaporated again; this procedure was repeated a third time with aqua regia, three times with hydrochloric acid, and three times with water.

The residue was taken up in several portions of water and the solution was filtered through a 5.5-cm. Whatman No. 42 filter paper into a 100-ml. volumetric flask containing 10 ml. of hydrochloric acid. For reassays and assays for parting acid losses, 50-ml. volumetric flasks containing 7 ml. of hydrochloric acid were used. The filter paper was kept for inclusion in the next reassay. Twenty milliliters of tin(II) chloride solution were added to 100-ml. flasks and 10 ml. were added to the 50-ml. flasks to form the colored complex. The mixture was diluted to volume and transmittance measurements were made on the Lumetron using either a 2-cm. or a 5-cm. cuvette. A blank which had undergone all fire and chemical treatments as above was prepared every time for each flux.

Test for Platinum in Parting Filtrate. A test for the presence of microgram amounts of platinum in large amounts of lead nitrate and nitric acid was developed on the lines of the niter assay.

In this type of assay a sulfide ore is fluxed in the presence of potassium nitrate. The amount of potassium nitrate used depends on the reducing power of the ore; some of the sulfur is oxidized by the nitrate and the remainder reduces litharge to form a lead button. The filtrate from the lead button parting was evaporated to dryness on the steam bath, and the lead nitrate crystals after being weighed were ground in a mortar to pass a No. 45 standard sieve. Sublimed sulfur was added to reduce the nitrate according to the following factor which was determined by experiment.

$$\text{Weight of sulfur} = \frac{6}{45.5} \times \text{weight of lead nitrate}$$

The factor for sulfur calculated stoichiometrically resulted in the reduction of too little litharge to give an adequate size of button. Added litharge to form the button was not required because of the lead in the lead nitrate. The above were rolled on a cellophane square with 3 grams of flour and 80% of flux 6; the whole was transferred to an assay crucible and covered with the remaining 20% of the flux. The pots were placed in the furnace at 975° C. and poured at 1150° C. The remainder of the procedure was the same as that described above under Nitric Acid Parting.

Standard Curves. In order to obtain standard curves for estimating the collection of platinum, it was considered necessary to make transmittance readings on solutions obtained from lead-platinum buttons which had undergone all operations except the actual fire assay collection. This was desirable because of the presence of many trace impurities which are collected during fire assay procedures. This requirement led to the development of the following procedure.

A number of blank lead buttons were obtained from each flux by the normal assay procedure. Each button was hammered flat and a cup was formed by turning up the edges. Standard platinum solution was added in varying amounts to cover the range desired, and taken to dryness in a steam cabinet. The lead was carefully folded, placed in the bottom of an assay crucible, covered with 20 grams of a mixture of 6 to 1 soda ash to silica sand, and heated to normal assaying temperatures. A portion cut from a steel pipe of large diameter and placed at the side of the mold was found useful as a shield to ensure quantitative recovery when these pots were poured. The rest of the procedure was the same as that described above. In addition, an assay was made on the parting solution and in this was included the fused soda ash–silica sand cover for the standard button. The aqua regia residues from both buttons were taken up with water and filtered into the same volumetric flask.

Standard working curves were plotted from the transmittance readings obtained. Slight variations in the slopes of the curves were noted for the different fluxes, but they were inappreciably different from the slope obtained by the use of pure solutions.

Sulfuric Acid Parting of Silver Beads after Cupellation. In the experiments involving prefusion and base metal additions the lead button was placed on a preheated bone ash cupel and cupelled at 900° C. The bead was placed in a 30-ml. beaker, 2 ml. of

Table IV Distribution of Platinum in Fire Assay

(Platinum taken, 4.99 mg.)

Flux	Flux No.	Button Weights, Grams: 1st	Parting acid assay	Reassay of parting acid	Reassay of slag from parting acid	2nd	Parting acid assay	3rd	4th	Grav. pt. in 1st button	Parting acid assay	Reassay of parting acid	Reassay of slag from parting acid	Platinum Found, Mg.: Benzenethiol filt. test	Total in 1st button	2nd button	Parting acid assay	Total in 2nd button	3rd button	4th button	Total Pt Recovered, Mg.	Pt Unrecovered, Mg.
Acid	2	25	29	27	30	25	29	27	23	4.283	0.133	0.122	0.008	0.000	4.546	0.101	0.025	0.126	0.028	0.005	4.71	0.28
		25	30	25	28	25	29	28	28	4.339	0.205	0.079	0.005	0.000	4.628	0.072	0.030	0.102	0.017	0.005	4.75	0.24
		27	27	28	29	26	27	28	25	4.436	0.135	0.149	0.002	0.001	4.723	0.015	0.015	0.030	0.012	0.002	4.77	0.22
Acid	3	27	28	26	29	30	27	30		4.737	0.093	0.051	0.000	0.003	4.884	0.007	0.000	0.007	0.000		4.89	0.10
		27	29	28	29	29	28	28		4.708	0.100	0.064	0.000	0.000	4.872	0.010	0.000	0.010	0.003		4.89	0.10
		26	27	27	29	26	28	27		4.723	0.105	0.070	0.000	0.000	4.898	0.005	0.000	0.005	0.000		4.90	0.09
Basic	4	30	28	28	30	25	29	21		4.894	0.046	0.030	0.004	0.000	4.974	0.033	0.015	0.048	0.007		5.03	−0.04
		29	27	28	29	24	28	20		4.803	0.063	0.042	0.007	0.000	4.915	0.032	0.008	0.040	0.007		4.96	0.03
		31	26	30	30	25	27	21		4.814	0.045	0.040	0.003	0.000	4.902	0.033	0.009	0.042	0.008		4.95	0.04
Neutral	5	27	26	31	29	32	23	24		4.738	0.070	0.082	0.003	0.000	4.893	0.048	0.035	0.083	0.008		4.98	0.01
		27	27	30	29	31	22	24		5.038	0.068	0.138	0.004	0.000	5.248[a]	0.040	0.010	0.050	0.008		5.31[a]	−0.32
		28	26	30	28	32	21	25		4.707	0.087	0.100	0.003	0.000	4.897	0.033	0.012	0.045	0.008		4.95	0.04
Neutral	6	28	26	31	27	31	23	23		4.703	0.080	0.060	0.001	0.002	4.846	0.115	0.017	0.132	0.002		4.98	0.01
		29	27	30	27	29	24	23		4.621	0.075	0.062	0.000	0.003	4.761	0.189	0.012	0.201	0.001		4.96	0.03
		28	27	27	27	29	22	21		4.616	0.085	0.059	0.004	0.001	4.765	0.148	0.013	0.161	0.001		4.93	0.06
Oxidizing	7	26	28	30	28	36	21	24		4.060	0.430	0.363	0.005	0.000	4.858	0.043	0.011	0.054	0.003		4.92	0.07
		26	29	29	27	37	18	25		4.453	0.243	0.255	0.000	0.000	4.951	0.025	0.010	0.035	0.003		4.99	0.00
		24	28	29	28	36	21	24		4.376	0.262	0.370	0.002	0.000	5.010	0.035	0.018	0.053	0.004		5.07	−0.08

[a] These numbers omitted from averages shown in Table VI.

concentrated sulfuric acid were added, and the contents were heated on a hot plate until the rapid evolution of bubbles had ceased. The beaker and contents were allowed to cool, 25 ml. of water were added, and the residue was recovered on a Whatman No. 42, 5.5-cm. filter paper. After being washed well with hot water, the filter paper was transferred to the original 30-ml. beaker and ignited overnight in an electric oven at 400° C. The remainder of the procedure involving aqua regia treatment was the same as that described above for nitric acid parting of lead buttons, and estimations of platinum were made from a standard curve.

Test for Platinum in Bead Parting Acid. The filtrate from the parting of the silver bead was evaporated on the steam bath to a small volume. The contents of the beaker were taken to dryness on a hot plate by fuming off the sulfuric acid. Aqua regia, hydrochloric acid, and water were added in the normal manner. Water was added to the nitric acid–free residue and the solution was filtered into a 50-ml. volumetric flask containing 7 ml. of hydrochloric acid. Tin(II) chloride solution (10 ml.) was added and the solution was made up to volume. Transmittance readings were made on the Lumetron using a 5-cm. cuvette. There was no case in which any trace of platinum was found in the bead parting acid. The above procedure was tested by additions of known amounts of standard platinum solution.

GRAVIMETRIC DETERMINATION OF PLATINUM IN LEAD BUTTONS

The benzenethiol procedure of Currah and others (*5*) formed the basis for the gravimetric determination of platinum in lead buttons. Base metals were removed by hydrolytic precipitation by a method adapted from a study by Gilchrist (*8*). This combination of methods led to a reliable procedure for platinum in lead buttons. Confirmation was obtained for the observation that the benzenethiol reagent must be stored in sealed glass vials because low results are obtained if it becomes oxidized.

Separation from Base Metals. The buttons containing 5-mg. amounts of platinum were parted as described above and the residue was finally treated with aqua regia, hydrochloric acid, and water. This residue was taken up in several portions of water and the solution was filtered through a 5.5-cm. No. 42 filter paper into a 50-ml. beaker. Two milliliters of 5% sodium nitrite solution were added and the solution was heated on the steam bath for approximately 1 hour. Twenty drops of 2% sodium bicarbonate solution were added and the sample was again heated on the steam bath for approximately 1 hour. Twenty drops of sodium bicarbonate solution were added and the mixture was allowed to cool. The small precipitate of base metals which formed was removed by filtration on a 5.5-cm., No. 42 filter paper, and the filtrate was evaporated to dryness several times with hydrochloric acid and several times with water. The residue was taken up in water and the solution was filtered into a 125-ml. conical flask; 1 drop of hydrochloric acid was added and the volume was made up to 60 ml. with water. One milliliter of benzenethiol reagent was added and the precipitate was allowed to coagulate on the steam bath for 2 hours. The mixture was allowed to cool and the precipitate was recovered on a 5.5-cm., No. 42 filter paper in a long-stemmed microfunnel. Any adhering precipitate was freed by means of a 1/8th sector of filter paper torn into several portions. The paper was folded into a tared Coors 00000 porcelain crucible and ignited to constant weight in an electric oven at 900° C. Deductions were made for blanks which had been treated in a similar manner. All filter papers were kept for inclusion in the reassays.

Test for Benzenethiol Filtrate Losses. The filtrate was evaporated to dryness on the steam bath and the residue was ignited by momentarily applying the flame of a Meker burner to the bottom of the beaker. Additions of aqua regia, hydrochloric acid, and water were made between evaporations in the normal manner. The aqueous solution of the residue was filtered into a volumetric flask containing hydrochloric acid and transmittance measurements were made with the Lumetron using a 5-cm. cuvette. Estimations of platinum losses were made by comparison with a standard curve prepared in a similar manner. Only in a few cases were traces of platinum found.

Results. Tables II, III, IV, and V show the experimental data obtained. The average per cent recoveries of the different flux types are compared in Table VI.

DISCUSSION

Tables III and IV indicate that there is no outstandingly successful type of flux for the over-all recovery of platinum. Acid

Table V. Effect of Base Metals on Recovery

Pt Taken, γ	Base Metal	Platinum Found, γ' — Normal Collection: 1st button	2nd button	3rd button	Total	Prefused Samples: 1st button	2nd button	3rd button	Total
100	None	90	..	..	90	75	..	..	75
500	None	500	..	..	500	400	..	..	400
100	Iron[a]	92	..	..	92	90	..	..	90
500	Iron[a]	498	..	..	498	389	..	..	389
100	Copper[b]	95	..	..	95	92	..	..	92
500	Copper[b]	490	..	..	490	405	..	..	405
100	Nickel[c]	46	26	1	73	60	14	11	85
500	Nickel[c]	340	92	7	439	245	122	36	403

Base Metal	Average % Recovery — Normal collection	Prefused samples
None[d]	95.0	77.5
Iron[d]	95.8	83.9
Copper[d]	96.5	86.5
Nickel[e]	80.4	82.8

[a] Flux prepared by adding 8.6 grams of Fe_2O_3 to flux 8.
[b] Flux prepared by adding 5.0 grams of CuO to flux 8.
[c] Flux prepared by adding 5.0 grams of NiO to flux 8.
[d] Values are for one assay only.
[e] Values are for one assay and two reassays.

Table VI. Comparison of Average Per Cent Recovery with Flux Type

		Flux No. 2	3	4	5	6	7
	Silicate degree	2.4	2.5	0.8	1.0	1.0	..
Assays on 5-mg. samples of Pt	1st button	92.6	97.7	98.6	97.9	95.8	98.8
	2nd button	1.7	0.2	0.9	1.2	3.3	0.9
	Total recovery	94.9	97.9	99.6	99.3	99.1	99.9
Assays of samples of 1 mg. and under	1st button	92.4	96.3	94.1	90.5	98.2	94.3
	2nd button	4.8	0.8	3.8	0.4	0.3	0.1
	Total recovery	97.2	97.1	97.9	90.9	98.5	94.4

flux 2, which has a high silica content and is viscous, gave the lowest recovery in the first button and required three reassays to give adequate collection. This retention of precious metal with high silica content was also noted in the cases of osmium, iridium, and palladium. Table VI shows that the average per cent recovery for fluxes 5 and 7 are better in the case of assays on 5-mg. amounts of platinum than for assays on samples of 1 mg. and under. A second assay of the parting acid was made for the 5-mg. amounts and it is possible that a similar assay for the 1-mg. additions would have resulted in closer agreement between the average recoveries.

Aside from occurrences in placers and primary deposits in olivene-rich rocks, magmatic nickel-copper–iron sulfide deposits make up the greatest known reserves of platinum metals (*9*). In this connection the results of the niter assays obtained with oxidizing flux 7 are particularly significant. One reassay gave good results with amounts of platinum up to 1 mg., and complete recovery was obtained for the 5-mg. additions. This type of flux also gave adequate results for rhodium, osmium, and palladium, but slag losses were noted for ruthenium and iridium.

In some of the previous investigations in this laboratory sulfide ores containing both copper and nickel were added to oxidizing fluxes to test the recoveries of platinum metals by niter assays. It was found during preliminary experiments that the presence of copper and nickel in a lead button resulted in large losses of platinum to the parting acid. Recovery of this platinum was possible by assaying the evaporated parting acid according to the method described above. However, it was decided to test the efficiency of the niter assay without complications caused by the presence of copper and nickel. Consequently sublimed sulfur instead of sulfide ore was added to oxidizing flux 7.

The facts that platinum was found in the parting acid and that the amount of platinum in the parting acid was increased when nickel and copper were present in the button are of the utmost significance in any proposed composite method of analysis for the platinum metals based on the parting of lead buttons. Possibly this loss is due to cell action, but some colloidal formation may also occur.

Flux 8 was used to determine the effect of large amounts of nickel, copper, and iron on the recovery of platinum. This flux was chosen because experiment showed that it was satisfactory for the assay of platinum metals in a high grade Falconbridge nickel-copper concentrate. Base metal additions corresponding to about 26% of copper and nickel and 40% of iron in 0.5 assay ton were made as shown at the foot of Table V. Because of the corrosive nature of this flux, the fusion time was reduced by placing the pots in the oven at 975° C. and removing them at 1125° C. Good recoveries of platinum were obtained for single assays in the presence of iron and copper, but nickel caused considerable losses even after two reassays. In the prefusion experiments performed with flux 8, lower recoveries of platinum were obtained in the presence of iron and copper in a single assay, but surprisingly, in the presence of nickel, the total recovery was slightly improved. Interference caused by nickel was very serious for iridium, but was small in the case of rhodium. Very similar recoveries of palladium and platinum in the presence of nickel (80 to 83%) were obtained.

Extreme differences in amounts of platinum recovered are to be noted in Table II. The fact that it was possible to account for all the platinum in acid fluxes 2 and 3 and that most of the platinum was unrecovered for flux 5 led to a further examination of the original pot in which this flux was prefused. As it was noticed that a small amount of liquid slag had crept up the walls of the pot, it was decided to subject all parts of the pot to an assay for platinum. The 20-gram assay crucible was broken into four vertical sections and these were placed inverted in a 100-gram pot. The charge (420 grams of flux 3 with added litharge and flour) was placed around and on top of the sections. Analysis of the resulting button (30.6 grams) showed the presence of 0.595 mg. of platinum.

With this evidence diverse results obtained in early prefusion experiments can be explained. Preliminary fusion of a flux salted with platinum solution results in migration of varying amounts of platinum, depending on the nature of the flux, to the pot walls. It would seem that certain types of fluxes encourage creeping of platinum up the walls of a pot in a manner somewhat analogous to the commonly observed creeping of a finely divided precipitate on the walls of a beaker. During normal reassaying, if the nature of the platinum present were similar, creeping might occur and the platinum would not be located where it could be effectively collected by reduced litharge. In the case of normal assaying where collection by lead can take place during the first fusion, the amount of platinum migrating to the pot walls would probably be small. However, this platinum would be difficult to recover by normal reassaying, especially if creeping occurred. The extreme differences in unrecovered platinum as shown in Table II strongly suggest that creeping beyond the meniscus of the flux is at a minimum with acid fluxes 2 and 3. The amounts of platinum that migrated to the pot walls for these fluxes were 78 and 55%, respectively.

Because of the success in recovering platinum from the walls of a broken pot, the same technique was used to test the walls of pots used for fusions in the presence of nickel. Table V shows that 61 and 97 γ of platinum were unrecovered from 500 γ additions to the normal and prefused samples, respectively. Assays of these pots using this technique yielded 5 and 9 γ of platinum, respectively. From this evidence it would appear that low recovery of platinum in the presence of nickel may be due to its loss to the slag.

Table VII. Cupellation of Lead-Platinum Buttons

(25-gram lead button)

Pt Taken, γ	Bead Size, Mg.	Platinum Found, γ In bead	In cupel	Total	Difference, γ
25	10	23	1	24	1
	75	25	0	25	0
	250	22	1	23	2
100	10	100	0	100	0
	75	98	0	98	2
	250	97	1	98	2
500	10	485	16	501	+ 1
	75	487	12	499	1
	250	500	0	500	0
1000	10	965	33	998	2
	75	981	20	1001	+ 1
	250	965	22	987	13

CUPELLATION OF LEAD-PLATINUM BUTTONS

Cupellation is the common process for the rapid determination of platinum in lead buttons. Palladium was the only one of the platinum metals investigated which was not subject to serious losses during cupellation. As the silver-platinum alloy forms readily, large losses were not expected to occur. The authors present below data on the behavior of platinum during cupellation.

Preparation of Lead Buttons. A cup-shaped container was made from a 5 × 5 inch square of lead foil, 0.005 inch thick. Varying amounts of standard platinum solution were added and evaporated to dryness in a steam cabinet. Weighed amounts of silver powder were added and the lead foil was carefully formed into a ball and wrapped with a 2 × 4 inch piece of lead foil. Synthetic lead buttons were made by high compression of the lead foil in a steel mold.

Procedure. The buttons were placed in the furnace on preheated bone ash cupels at a temperature of 900° C. and left until the lead was removed and the silver beads had formed. Parting in sulfuric acid and estimation of platinum were made as described above. No trace of platinum was found in the sulfuric acid parting solution by the method described above.

Assay of Cupels. The used cupel was assayed to determine whether any mechanical or absorption losses of platinum had occurred. The stained part only was weighed and ground in a mortar to pass a No. 45 standard sieve. The following additions were made to flux the stained bone ash: 3/4 of its weight of soda ash, 1/3 of its weight of borax glass, 1/4 of its weight of calcium fluoride, 1/6 of its weight of silica sand, and excess flour (4 grams). These substances were intimately mixed by rolling on a cellophane sheet and were placed in an assay crucible. Fusion temperatures were 1000 to 1140° C. The lead button was parted in nitric acid (1 to 4) and platinum was determined colorimetrically. Comparisons were made with a standard curve prepared by assay of cupels stained by cupelling salted lead foil without added silver.

Table VII indicates that no significant losses of platinum occurred during cupellation. Difference in the bead size did not appear to be an important variable, but larger losses to the cupel were recorded when the ratio of platinum to silver was reduced to 20 to 1 and 10 to 1. The results with these small beads (10 mg.) are of interest because they are used in spectrographic determinations of the platinum metals in ores and concentrates.

CONCLUSIONS

The distribution of platinum during the various processes involved in a fire assay has been examined. Acceptable over-all recoveries were obtained except where the slags contained considerable nickel. Nearly complete recovery was obtained with only one reassay, except with acid fluxes, where high silica content tended to hinder the collection. Pot wall loss may be a major reason for the small amounts of platinum not recovered in normal fire assaying. Serious loss of platinum to the nitric acid parting solution was experienced in some cases. Cupellation losses with platinum were not significant.

ACKNOWLEDGMENT

Appreciation is expressed to the Canadian Department of Agriculture, Science Service, for financial support and leave of absence given to I. Hoffman.

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RECEIVED for review December 22, 1955. Accepted March 5, 1956.

Modified Micro-Dumas Procedure for Determining Nitrogen

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A modified Dumas procedure has been developed in which the small movable burner is replaced by a regular furnace. The advantages are: more complete combustion, a shorter burning time, and a minimum of handling by the operator.

RECENT improvements in the Dumas method for the microdetermination of nitrogen in organic compounds have included the use of nickel oxide, high temperatures, oxygen, mixing chambers, etc. (*1*, *3–5*). Another modification, developed in this laboratory, substitutes a micro combustion furnace for the movable burner.

EQUIPMENT

From left to right the apparatus consists of the usual nitrometer, two A. H. Thomas Co. micro combustion hinged-front furnaces (Catalog No. 5678-A) on a slightly enlarged stand with the stationary furnace on the left and the burner furnace on the right, and a Poth-type carbon dioxide generator (*2*). A standard Vycor combustion tube is used with a permanent filling of copper oxide, reduced copper, and copper oxide, in that order. The stationary furnace covers the permanent filling. The temporary filling, which includes the mixture of fine copper oxide and sample, is added to the tube so that the sample is located in the area to the left of the center of the burner furnace—that is, it should be enclosed within 4 to 10 cm. of the left end of the burner furnace; otherwise, the unburned sample might possibly sublime away from the heat. The tube is then placed on the stand so that it extends 5 cm. beyond the edge of the stand towards the nitrom-

Table I. Nitrogen Determinations

Compound[a]	% Theory	% Found
Sulfanilic acid	8.09	8.06 8.13
Acetanilide	10.36	10.35 10.51
Nicotinic acid	11.35	11.35 11.30
Cystine	11.66	11.46 11.52
Benzylisothiourea HCl	13.82	13.88 14.03
Azobenzene	15.38	15.37 15.40
2,4-Bis(benzylamino)6,7-diphenylpteridine	16.99	17.11 16.96
4-(4-Morpholinyl)-6,7-diphenylpteridine	18.96	19.00 18.83
p-Carboxyphenylazobarbituric acid	20.30	20.45 20.32
2,4-Bis(3-diethylaminopropylamino)-6,7-diphenylpteridine	20.72	20.75 20.60
2,4-Bis(2-hydroxyethylamino)6,7-diphenylpteridine	20.88	20.90 20.96
Sulfadiazine	22.39	22.28 22.34
2,4-Bis-allylamino-6-(*p*-chloroanilino)-*s*-triazine	26.61	26.89 26.61
Arginine HCl	26.60	26.62 26.74
2,4-Diamino-6,7-diphenylpteridine	26.74	26.80 26.79
Tetramethyldipyrimidopyrazinetetrone	27.62	27.54 27.57
4-Amino-6-hydroxy-5-nitropyrimidine	35.90	36.03 35.90
Thioguanine	41.92	42.23 41.94

[a] Sample weights between 3 and 5 mg.

eter. The furnace temperatures are close to 675° C. for the stationary furnace, and 725° C. for the burner furnace. If numerous halogen or sulfur compounds are run, the tube should be burned out or replaced. Old used tubes should be discarded.

PROCEDURE

A modified Dumas procedure is used. The combustion tube is connected to the nitrometer and the carbon dioxide source and flushed out with carbon dioxide for a few minutes. The stationary furnace is pulled over the permanent filling and when microbubbles are obtained, the carbon dioxide is stopped. The burner furnace is slowly drawn from the side across the tube over the temporary filling, depending upon the bubble rate (not more than 3 per second), and finally placed entirely over the tube and butted against the stationary furnace. When the bubble rate diminishes to 1 every 5 seconds, the carbon dioxide is turned on and the bubble rate is adjusted to 4 per second. The furnaces remain in position until near-microbubbles appear, then are moved back. When microbubbles are obtained, the determination is complete. The time taken for an analysis usually runs between 15 and 20 minutes.

DISCUSSION AND RESULTS

This particular modification was developed after considerable experimentation with various high temperature movable burners, etc. The advantages that appeared were several: more complete combustion, a shorter burning time, and a minimum of handling by the operator. There is no need for reburning, as the furnace covers the entire sample and temporary filling. There is less possibility of burning the sample too fast, thereby having incomplete combustion, because the whole tube is maintained at a rather high temperature. Once the furnaces are in place—that is, over the tube—the apparatus requires very little attention. It is simply a matter of adjusting the carbon dioxide flow and terminating the procedure when microbubbles appear.

To test the efficiency of this modification a variety of compounds were analyzed, as shown in Table I. In addition, hundreds of routine samples have been run with very good results.

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RECEIVED for review September 30, 1955. Accepted March 21, 1956.

Infrared Absorption Spectra of Branched-Chain Fatty Acids

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From a study of the infrared absorption spectra of long branched-chain fatty acids Freeman has shown that the relative intensities of the bands at 7.8 and 8.1 microns are valuable in identifying α-substitution. This correlation holds for the branched-chain hexanoic acids. In addition, the relative intensities of the bands at 6.8 and 7.1 microns are valuable in identifying α-substitution in acids containing less than 14 carbon atoms.

FREEMAN (*1*) in his study of the infrared spectra of 27 branched long-chain fatty acids found that the relative intensities of the absorption bands near 7.8 and 8.1 microns could be used to distinguish fatty acids with a branched-chain in the α-position. The band at 7.8 microns was the stronger of the two, except when a group was substituted in the α-position.

To see whether this correlation would hold for the branched short-chain fatty acids, the spectra of 15 such acids were measured on a Perkin Elmer Model 21 double-beam recording infrared spectrometer equipped with rock salt optics. The liquid acids were run in a demountable liquid cell without dilution. No spacer was employed.

The fatty acids were synthesized by the Bureau of Mines and were obtained from the Chemical Corps Chemical and Radiological Laboratories. The position of branching was determined by the method of synthesis. Carbon-hydrogen analysis and neutralization equivalents were reported. Agreement between the calculated and experimental values was excellent. Freezing point data were used to determine mole per cent purity in several cases. The 2-isopropyl; 2-*n*-butyl-, 3-*n*-propyl-, 4-ethyl-, and 5-methylhexanoic acids were better than 95 mole % pure. The 2-ethyl- and 3-methylhexanoic acids were, respectively, 92 and 89% pure.

The spectra of these acids in the region of 6.5 to 8.5 microns are plotted in Figure 1. It is apparent from this figure that the

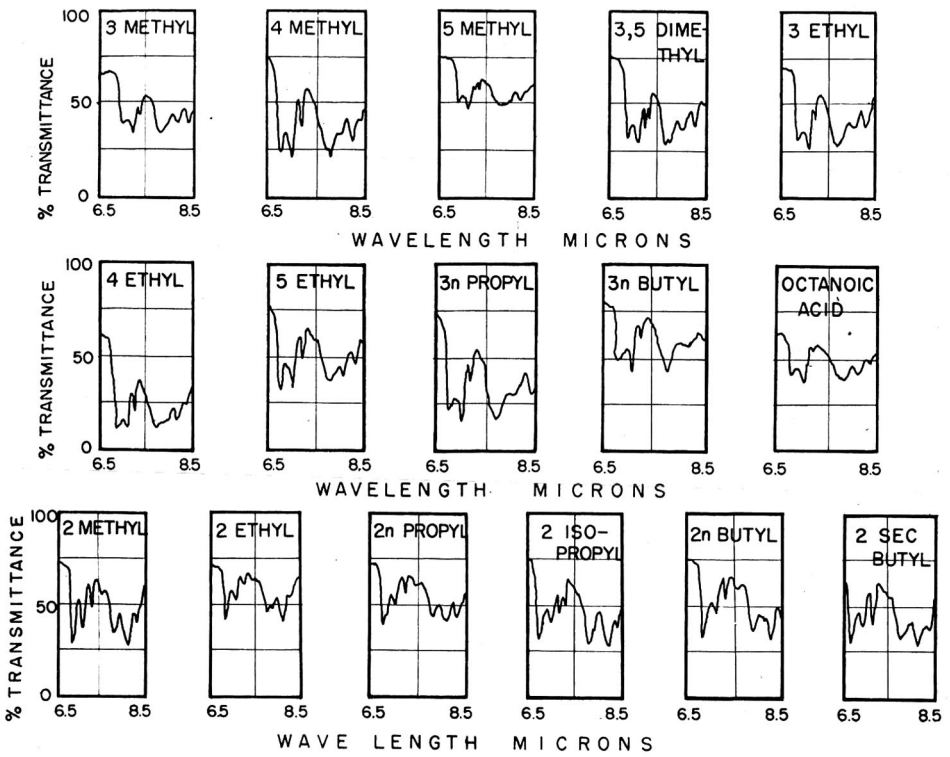

Figure 1. Spectra of various hexanoic acids and octanoic acid in the region of 6.5 to 8.5 microns

Table I. Observed Wave Lengths (Microns) of the Absorption Bands Near 8.1 Microns in Two Series of Methyl Branched-Chain Fatty Acids

Acid	Wave Length, μ	Acid	Wave Length, μ
2-Methylhexanoic	8.10	2-Methyloctadecanoic	8.09
3-Methylhexanoic	8.13	3-Methyloctadecanoic	8.14
4-Methylhexanoic	8.20	4-Methyloctadecanoic	8.23
5-Methylhexanoic	8.27	5-Methyloctadecanoic	8.25

Table II. Observed Wave Lengths of Absorption Bands of Branched-Chain Fatty Acids between 12.5 and 14.0 Microns

Acid	Wave Length, μ				
2-Methylhexanoic	12.66	...	...	...	13.76
3-Methylhexanoic	...	...	...	13.56	...
4-Methylhexanoic	...	12.88	...	...	...
5-Methylhexanoic	...	...	13.40	...	...
3,5-Dimethylhexanoic	...	...	...	...	13.85
2-Ethylhexanoic	...	12.82	...	...	13.76
3-Ethylhexanoic	...	12.92	...	...	13.61
4-Ethylhexanoic	...	13.02	...	...	...
2-*n*-Propylhexanoic	12.65	...	...	13.50	...
2-Isopropylhexanoic	12.63	...	...	...	13.69
2-*n*-Butylhexanoic	12.65	...	...	...	13.68
2-*sec*-Butylhexanoic	12.75	...	...	...	13.69
3-*n*-Propylhexanoic	...	...	...	13.56	...
3-*n*-Propylheptanoic	...	...	...	13.55	13.70
5-Methylheptanoic	...	12.95	...	...	...
Octanoic	...	...	...	...	13.83

band near 8.1 microns is stronger than the band near 7.8 microns only when α-substitution occurs.

In addition to these two bands, the relative intensities of the bands at 6.8 and 7.1 microns are useful in identifying α-substitution. Absorption at 7.1 microns is stronger than absorption at 6.8 microns, except when α-substitution occurs. However, because the 6.8-micron band intensity is a function of the size of the hydrocarbon portion of the molecule, at a chain length of approximately 14 carbon atoms the 6.8-micron band becomes stronger than the 7.1-micron band, regardless of the type of branching. Hence, this intensity inversion of the 6.8- and 7.1-micron bands is applicable only to the short-chain fatty acids and is not as generally useful as the 7.8- to 8.1-micron region initially investigated by Freeman.

It is also of interest to compare the position of the band near 8.1 microns in the methyl hexanoic acid series with those in the methyl octadecanoic acid series as reported by Freeman (see Table I).

Table I shows that the band positions may be correlated with position of branching in the methyl-substituted series. In the ethyl hexanoic acid series absorption occurs at 8.15 microns in both the 2-ethyl- and 3-ethylhexanoic acids and at 8.25 microns in the 4-ethylhexanoic acid. Changes in the position of the absorption band near 7.8 microns were not significant.

Freeman also reported spectral indications for ethyl and *n*-propyl groups. The ethyl group is associated with a band at 12.95 microns and the *n*-propyl group with one at 13.5 microns. Table II shows that these correlations are confirmed by the present work.

The isopropyl group may be recognized by absorption near 7.30 microns in the three fatty acids containing this group, as has been shown by Freeman and Sobotka and Stynler (*2*).

This investigation shows that the correlations presented by Freeman may be extended to include the lower fatty acids. The relative intensities of the bands at 6.8 and 7.1 microns serve to identify α-substituents in acids containing less than 14 carbon atoms.

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RECEIVED for review November 16, 1955. Accepted March 19, 1956. This study was conducted under contract between the Chemical Corps, U. S. Army, and Rensselaer Polytechnic Institute.

Improved Technique for Two-Dimensional Circular Paper Chromatography

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The segment-sector combination is presented as an improvement over the ring-disk technique in two-dimensional circular paper chromatography. This new technique seems to give better results with amino acids than could be obtained using the conventional two-dimensional circular chromatograms.

CIRCULAR (disk) paper chromatography is now being used on an increasing scale (*2, 3, 8, 10-12*). A method for two-dimensional circular paper chromatography (*1*) has been applied to the separation of certain organic acids (*5, 6*). This application and observations regarding the path of a substance in the "ring" during the first run (*4*) led the present authors to attempt to develop a "combination" which would give a more distinct separation of the arcs themselves rather than of merely the outer tips forming the spiral (*1*). It was hoped that this would limit the rather extensive spreading of the arcs in such a way as to give only slightly elongated spots.

EXPERIMENTAL

Definitions. RING. The ring of a filter paper, external diameter 6 cm., width 0.3 cm., opening into a tail.

SEGMENT OF RING. Suitable portion of a circular ring, external diameter 9 cm., width 0.5 cm.

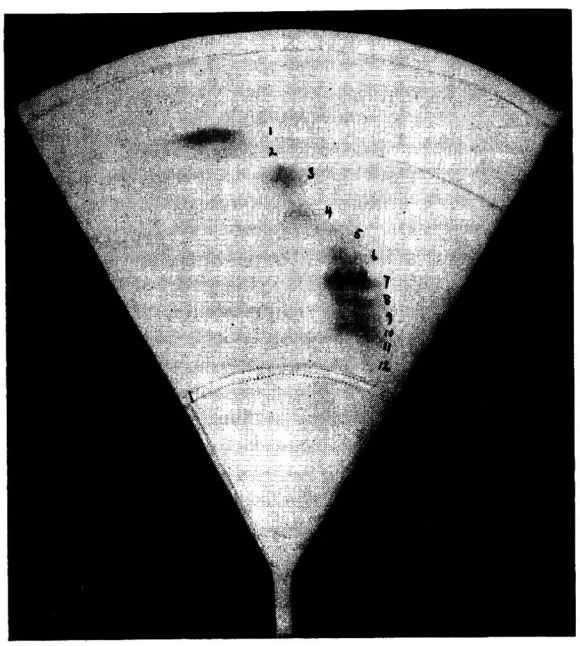

Figure 1. Two-dimensional circular paper chromatogram using segment-sector combination

COMBINATION. A filter paper disk or its sector, on which a ring or a segment, after the first run, is stitched.

SECOND RUN. Development of the combination.

Materials and Reagents. Whatman No. 1 filter paper, Partridge's solvent (*9*); 0.4% ninhydrin solution in aqueous acetone for use as spray reagent.

Apparatus. Teakwood chamber (outer measurements, 40 × 40 × 10 cm.) with glass slide supports along the inside circumference, on which the paper disk rests; the interior of the box is coated with wax.

Micrometer syringe, Agla (Burroughs, Wellcome & Co., London, England), for spotting.

The first step in this investigation was to determine the manner in which a substance spreads itself in the ring during the first run. This was done by applying phenylalanine solution to six rings and developing them in separate chambers so that any one of the rings could be removed as soon as the solvent front had reached a desired point on the ring. After spraying with ninhydrin, the shape developed by the amino acid when it had traversed the given distance in the ring could be noted. The amino acid showed a sickle shape when it had traversed the maximum distance in the ring, the advance point curling inwards, the tail end thinning out into the solvent, and the middle portion (which touched the outer rim of the ring) showing the maximum color density. Such a far-flung spot in the ring would obviously give, in the combination, an arc even more extensively spread out, as it does in practice. In spite of its sickle shape in the ring, this arc is fairly uniform, a point which prompted certain other experiments.

A single amino acid was applied at three adjacent points which were at slightly unequal distances from the source of the solvent. For this experiment phenylalanine, alanine, glycine, and cystine were selected. After the development of a circular as well as a strip paper chromatogram, it was found that the distances of the points of application from the solvent source for phenylalanine did not show a significant difference. The three points of application of this amino acid seemed to have behaved as parts of a single somewhat elongated point of application of the amino acid. In the case of cystine, however, the effect of this inequality of distances was clearly marked. This is in agreement with general findings that the R_f values of amino acids are affected by the distances between their starting points and the source of the solvent (*7*).

DISCUSSION

When a segment was used for the first run instead of a ring, no sickle shapes were observed. Somewhat arrow-shaped spots appeared instead, thereby minimizing the possibility of overlapping. Obviously, when the segment replaced the ring in the combination, the source of the solvent had to be somewhere near the periphery of the disk rather than at the center. Such an arrangement would secure a radial flow of the solvent with respect to all the amino acids only if, instead of a disk, a suitable sector of the disk were used, allowing its apex to serve as the wick. Therefore, a combination was manipulated of a segment which, after its first run, was stitched onto a sector of a filter paper disk in such a way that the two sides of this sector were at right angles to the tangents at the two ends of the segment. The radius of this sector was 24 cm. and the straight distance between the two ends of the segment was 9.3 cm. The time required for development was 24 hours.

However, the time required for development of the segment-sector combination could be reduced if the wick end of the sector was padded by sandwiching it between two filter paper sectors whose arcs reached to within 1.2 cm. of the inner rim of the segment. Under these conditions the time required for the development was approximately 7 hours (Figure 1).

The segment-sector combination appears to give more satisfactory results than the ring-disk combination (*3*); yet it maintains the distinctive feature of circular paper chromatography—i.e., to let the flow of the solvent be radial with respect to the point of application of a substance. Moreover, it ensures a better separation of amino acids as compared to the ring-disk combination. Furthermore, by limiting the extent of the arcs

to only slightly elongated spots, it promises to lend itself to quantitative examination by a densitometer.

At the present stage of its development, two-dimensional circular chromatography can hardly compete with the conventional two-dimensional chromatography. However, it is hoped that the findings reported here may lead to further improvements in this technique.

ACKNOWLEDGMENT

J. W. Airan thanks the Sir Dorabji Tata Trust, Bombay, India, and the Asia Christian Colleges Association, London, England, for financial assistance. Both the authors thank Lewis Simmons, Wilson College, Bombay, India, for helpful suggestions.

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RECEIVED for review October 25, 1955. Accepted March 27, 1956.

Colorimetric Standard for Carotene

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Solutions of β-carotene in purified mineral oil show a fairly constant absorptivity at 436 mμ under normal laboratory conditions for at least 9 months. These solutions may be used as calibration standards for the determination of carotene and for interlaboratory collaborative work.

AN AQUEOUS solution of Naphthol Yellow and Orange G has been recommended by Sprague (*14*) for use as a colorimetric standard for carotene, while azobenzene solutions have been used advantageously by Kuhn and Brockmann (*7*). The popular method of Guilbert (*6*) for determining the provitamin A value of forages employed a slight modification of the Sprague color standard. However, potassium dichromate has probably been utilized most frequently as the colorimetric standard in carotene determinations (*1, 3, 8, 13*). Munsey (*9*) prepared a standard curve from carotene solutions for use in a neutral wedge photometer. He found variations caused by the color standards and the spectrophotometer, in the results of the determination of carotene, and attempted to obtain collaborative results on a carotene standard in coconut oil. The carotene was not stable in this solvent and bleached too rapidly to be an effective standard (*10*).

There is a need for a stable β-carotene solution which can be utilized as a colorimetric standard in interlaboratory collaborative studies. β-Carotene in purified mineral oil solutions exhibited almost complete stability at the range of concentrations suitable for spectrophotometric determinations. At 436 mμ there was no significant change in the absorbances of the mineral oil solutions of β-carotene for a period of approximately 9 months.

REAGENTS AND APPARATUS

β-Carotene. Pure, crystalline β-carotene was prepared by the procedure of Bickoff and others (*2*). It had an absorptivity of 256 at 451 mμ in commercial hexane.

Mineral Oil. A medicinal grade of light, white mineral oil (Sherwood Refining Co., Englewood, N. J.) was purified and degassed by passing through a column (75 × 250 mm.) of alumina (Merck & Co.) under reduced pressure.

Commercial Hexane, Skellysolve B. Purified by passing through a large column of silica gel and distilled over solid potassium hydroxide.

Acetone. Reagent grade, passed through anhydrous sodium sulfate and distilled over mossy zinc.

Benzene. Distilled over solid potassium hydroxide.

A Beckman Model DU spectrophotometer with matched 1-cm. cells was used for all absorbance measurements.

EXPERIMENTAL

The absorptivity of β-carotene in both mineral oil and hexane was calculated in the following manner. A solution was prepared by dissolving a weighed amount (approximately 3 mg.) of the crystalline material in 3 ml. of benzene in a 1-liter volumetric flask and diluting to volume with mineral oil or hexane. The absorbance was determined at 5-mμ intervals except in regions of maximum absorbance, where readings were taken at 2-mμ intervals. The absorptivity, a, was calculated from the equation

$$a = \frac{A}{bc}$$

in which A is the absorbance, b, the cell length in centimeters, and c, the concentration in grams per liter.

The stability of carotene in mineral oil over a 9-month period was determined under several storage conditions. Under the first set of conditions, the solution was prepared with no prior purification of the oil and no subsequent removal of the benzene used to dissolve the carotene. These solutions were stored in the dark at room temperature. Another set of conditions involved purification of the mineral oil by passing through alumina prior to dissolution of the carotene, and subsequent removal of the volatile benzene by holding the sample under a vacuum (0.1 mm. of mercury) with occasional stirring for 24 hours. Portions of such solutions were stored in the dark at room temperature in 3-gram screw-cap vials. Other portions were sealed in soft glass ampoules under a vacuum (1 to 2 microns of mercury) and stored at room temperature as well as at 7° C. The absorbances of these solutions were determined periodically during the storage period.

Table I. Effect of Solvents on Wave Length of Maximum Absorption and Absorptivity of β-Carotene

Solvent	First Maximum		Second Maximum		436 mμ,
	Mμ	a	Mμ	a	a
Hexane[a]	479	225	451	256	196
Mineral oil[a]	487	197	459	229	165
Acetone-hexane[a] (10 and 90, v./v.)	480	222	452	252	191

[a] Contained 0.3% benzene.

RESULTS AND DISCUSSION

The absorptivities of β-carotene in mineral oil were compared with the absorptivities in 10% acetone in hexane because carotene probably is determined most frequently in the latter solvent (*4, 5, 11, 12*). The absorptivities exhibited some variation in different solvents, as did the wave length of maximum absorbance (Figure 1, Table I). The shape of the absorption curve in mineral oil was almost identical to that in hexane or 10% acetone solu-

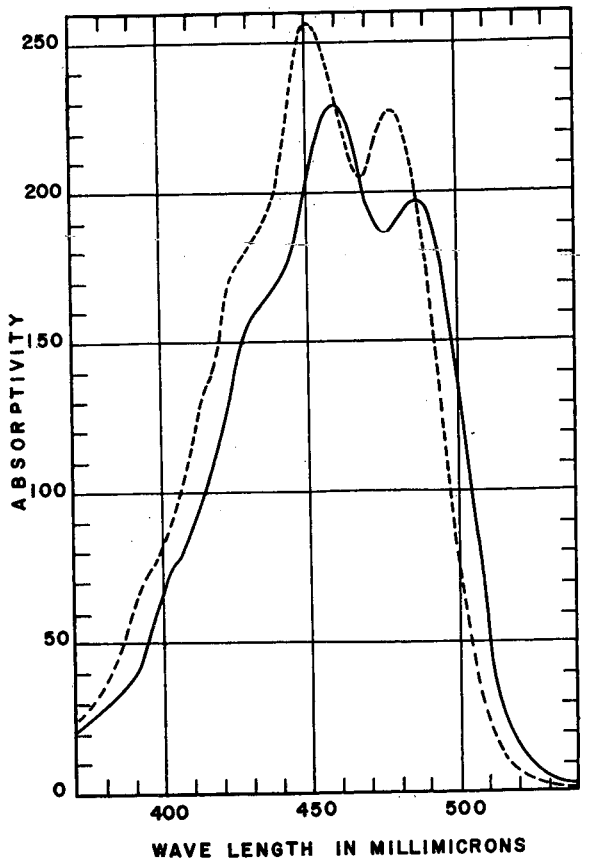

Figure 1. Comparison of absorptivity spectra of β-carotene

———— Mineral oil (0.3% benzene)
- - - - - Hexane (0.3% benzene)

Table II. Stability of β-Carotene in Mineral Oil Stored 9 Months in Dark

Conditions	Absorbance at 436 mμ Initial	Final
Mineral oil not degassed, benzene not removed; stored in vials at room temperature	0.612 0.370	0.595 0.360
Mineral oil degassed, benzene removed; stored in vials at room temperature	0.378	0.376
Sealed ampoule, stored at 7° C.	1.60	1.60
Sealed ampoule, stored at room temperature	1.60	1.60

tions of hexane, with about an 8-mμ shift to longer wave lengths (Figure 1). The absorption spectrum in 10% acetone–90% hexane is shifted about 1 mμ toward longer wave lengths from that in hexane solutions.

The absorbances of mineral oil solutions indicated essential stability at 436 mμ (Table II). The absorbances at the wave lengths of maximum absorbance (487 and 459 mμ) decreased somewhat with time and shifted slightly (1 mμ) toward the ultraviolet, even when the absorbance at 436 mμ remained unchanged. Probably this was a manifestation of some spontaneous trans to cis isomerization of the β-carotene. The solutions apparently were as stable at room temperature in screw-cap vials as they were sealed under high vacuum in glass ampoules. Complete stability was not achieved when the mineral oil was not purified and degassed before dissolving the β-carotene (Table II).

Because β-carotene exhibits stability at 436 mμ in mineral oil solutions and because it probably is determined most frequently at that wave length (*11*), it should be possible to utilize mineral oil solutions of β-carotene as standard solutions. There is adequate stability for interlaboratory collaboration and for use in

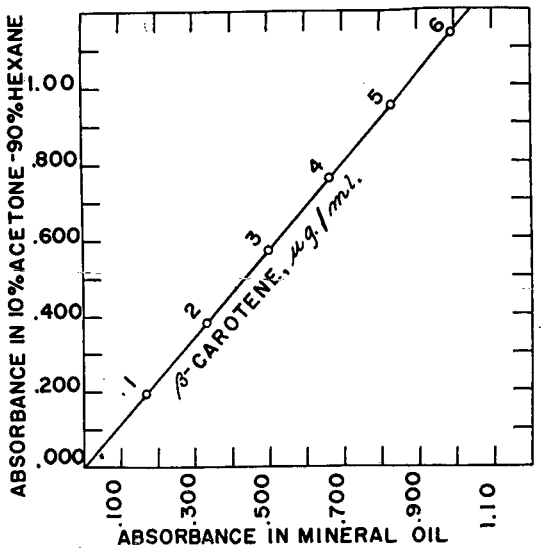

Figure 2. Curve for conversion of absorbances of equivalent concentrations of β-carotene in mineral oil at 436 mμ and in 10% acetone–90% hexane

instrument calibration for the particular determination of carotene. Mineral oil standard solutions can be sealed in ampoules without danger of fire or loss of solvent. Stored in this manner they probably would be stable almost indefinitely if they were protected from strong light. However, it is apparent that storage in screw-cap vials is also entirely satisfactory.

Because the absorptivity of β-carotene in mineral oil is less than in 10% acetone–90% hexane solutions, a conversion curve may be used to obtain equivalent absorbances (Figure 2). These solutions obey Beer's law as do other solutions of β-carotene.

ACKNOWLEDGMENT

The authors are deeply appreciative of the suggestions of F. W. Quackenbush in promoting this study.

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RECEIVED for review January 9, 1956. Accepted March 29, 1956. Journal Paper No. 937 of the Purdue Agricultural Experiment Station, Lafayette, Ind.

Contents Page Omission

Through a regrettable error, no listing was made on the contents page of the June issue of the article on "Semiquantitative Spectrochemical Analysis of Silicon" by P. H. Keck, A. L. MacDonald, and J. W. Mellichamp, which was printed on page 995.

Amperometric Determination of Zirconium with 1-Nitroso-2-naphthol

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An amperometric method has been developed for the determination of zirconium, based on the interaction of this element with 1-nitroso-2-naphthol. This method can be used for the accurate determination of zirconium in the presence of small amounts of fluoride ion; however, large concentrations of fluoride ion interfere. The amperometric titration of zirconium in the presence of several other diverse ions indicated that only nickel interfered with the titration. The average relative analytical error of the method, corresponding to a tenfold change in concentration of zirconium, is ±0.4%. The method is rapid and involves only a few operations.

ALTHOUGH numerous precipitation reactions of zirconium have been reported (*1*), it appears that only one of these reactions has been utilized for the amperometric determination of zirconium. This is the titration of zirconium with cupferron (*3*) in sulfuric acid solution, which was found to be applicable for the determination of this element in fluoride solution. The analytical precision of the method was reported to be 0.4% or better.

Wilson and Lovelady (*6*) have studied the amperometric titration of iron(III) with 1-nitroso-2-naphthol in acetic acid–sodium acetate buffer. Kolthoff and Langer (*2*), while investigating the amperometric titration of cobalt with 1-nitroso-2-naphthol, observed from a few experiments that palladium and copper could also be titrated with the same reagent. Zirconium salts (*5*) react with 1-nitroso-2-naphthol solution to form a greenish yellow amorphous precipitate of the composition $C_{10}H_6O(NO)_2ZrO$. However, zirconium reacts with 1-nitroso-2-naphthol in acetate buffer to form a dark brown precipitate. Also, when the pH is less than 5, zirconium acetate solutions of high ionic strength show no apparent tendency to form hydrous oxide of zirconium; this fact suggests that zirconium probably exists in solution as a stable complex or colloid.

The present investigation was undertaken to study amperometrically the zirconium–1-nitroso-2-naphthol reaction in acetate buffer, to develop a method for the rapid determination of small amounts of zirconium, and to determine the effect of certain diverse ions on the titration.

EXPERIMENTAL

Reagents and Solutions. A weighed amount of C.P. zirconyl chloride octahydrate (A. D. Mackay, Inc.) was dissolved in distilled water and diluted to volume to give the concentration desired. This solution was standardized gravimetrically by treating aliquots of the stock zirconium solution with ammonium hydroxide according to the procedure described by Scott (*4*); the precipitate was ignited and weighed as zirconium dioxide. The average of quadruplicate determinations of zirconium in this manner was 12.3 mg. per ml., with an average deviation of ±0.01 mg. per ml. 1-Nitroso-2-naphthol solution was prepared after recrystallizing this reagent (Eastman Kodak Co., No. p428) twice from alcohol, and the solution was standardized amperometrically (*6*). A buffer solution was prepared which was 2*M* in both acetic acid and sodium acetate. All other reagents were the same as those described by Wilson and Lovelady (*6*).

Apparatus. A Sargent Model XXI Polarograph was used. The H-type polarographic cell contained a saturated calomel electrode and a potassium chloride–agar–fritted-glass disk salt bridge; the entire assembly was jacketed in water at 25° ± 0.1° C. All measurements were made and are reported *vs.* the saturated calomel electrode at 25° C.

Procedure. After a preliminary study of the zirconium–1-nitroso-2-naphthol system, the following procedure was adopted for the amperometric determination of zirconium.

An aliquot of the standard stock solution of zirconium, sufficient to give the final concentration desired, was placed in a 100-ml. volumetric flask. Then 4 ml. of 0.2% gelatin solution, 5 ml. of 2*M* acetate buffer, and 5 ml. of 25% potassium chloride (to maintain a high constant ionic strength during the titration) were added, and the solution was diluted to volume with distilled water. A 20-ml. aliquot of this solution was transferred to an H-type cell, and oxygen-free nitrogen (*6*) was passed through the solution for 15 minutes. All current measurements were taken at −0.4 volt and were corrected for dilution effects. This solution was titrated amperometrically with standard 1-nitroso-2-naph-

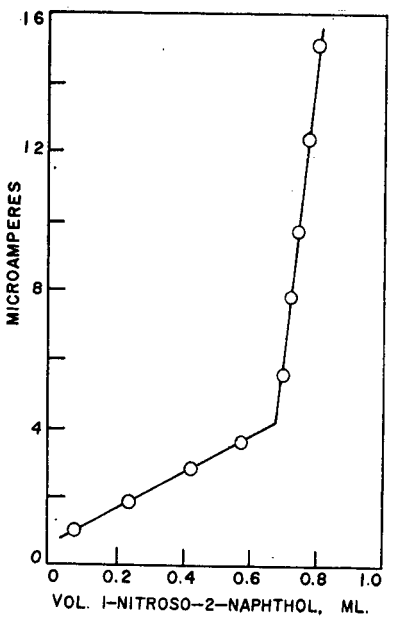

Figure 1. Titration of 20 ml. of 0.150 × 10^{-2} *M* zirconium with 0.0930*M* 1-nitroso-2-naphthol

Table I. Amperometric Titration of Zirconium in Acetate Buffer at −0.4 Volt *vs.* S.C.E.

Zirconium, Mmoles × 10^2	1-Nitroso-2-naphthol, Mmoles × 10^2	Mole Ratio, Zr/Titrant
0.40	0.80	1:2.00
1.20	2.40	1:2.00
2.00	4.05	1:2.03
2.40	4.81	1:2.00
3.00	6.03	1:2.01
4.00	8.00	1:2.00
5.00	9.99	1:2.00

Table II. Amperometric Titration of Zirconium in Presence of Diverse Ion

(0.0240 mmole of zirconium taken)

Added			Zirconium, Mmole	
Ion	Mmole	Form	Found	Dev.
Ni(II)	0.02	$NiCl_2.6H_2O$	0.0229	−0.0011
Ti(IV)	0.02	$TiCl_4$	0.0242	+0.0002
Al(III)	0.02	$AlCl_3$	0.0239	−0.0001
Ca(II)	0.02	$CaCl_2.2H_2O$	0.0237	−0.0003
Zn(II)	0.02	$ZnCl_2$	0.0238	−0.0002
Cr(III)	0.02	$CrCl_3.6H_2O$	0.0242	+0.0002
Mg(II)	0.02	$MgCl_2.6H_2O$	0.0238	−0.0002
Au(III)	0.01	$AuCl_3.HCl.3H_2O$	0.0241	+0.0001
SO_4^{--}	0.20	K_2SO_4	0.0239	−0.0001
NO_3^-	0.20	KNO_3	0.0240	0.0000
F^-	0.25	$KF.2H_2O$	0.0241	+0.0001

thol. The volume of titrant used in each titration was determined using the extrapolation method.

A typical titration curve for the amperometric determination of zirconium is shown in Figure 1. The data obtained from several titrations of zirconium with 1-nitroso-2-naphthol are shown in Table I; each line in the table is the mean of two results obtained from the titration of solutions of the concentration indicated.

Effect of Diverse Ions. The possible interference of several ions on the amperometric titration of zirconium in acetate buffer was studied by adding the diverse ions, individually, to solutions which were approximately 1 mM in final zirconium concentration. These solutions were made according to the adopted procedure and the volume of titrant used in each titration was determined in the usual way. The results of these titrations are shown in Table II.

DISCUSSION

The average relative analytical error of this method is ±0.4%, which corresponds to about a tenfold change in concentration of zirconium. Results of the titration of zirconium in the presence of small amounts of diverse ion indicate that the ions selected for study, with the exception of nickel, did not interfere in the determination of zirconium. The interference of copper, cobalt, iron, and palladium on the titration was not studied because these elements are readily precipitated by 1-nitroso-2-naphthol.

Zirconium was titrated accurately in the presence of a 10 to 1 mole ratio of fluoride ion to zirconium. However, a large concentration of fluoride ion interfered—i.e., the volume of titrant required to titrate zirconium in the presence of a 100-fold molar excess of fluoride ion was too large by about +4%.

The analytical precision of this method is comparable to that of the cupferron procedure (*3*); the 1-nitroso-2-naphthol titrant is stable for 2 weeks as compared to 1 day for the cupferron titrant.

ACKNOWLEDGMENT

The work reported in this paper was made possible by a grant from National Science Foundation.

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RECEIVED for review August 8, 1955. Accepted March 30, 1956.

Separation and Purification of Milligram Amounts of Cesium from Large Amounts of Other Alkali Salts

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A procedure is presented for the separation of milligram quantities of cesium from approximately ten thousand times as much sodium and five times as much rubidium. At the same time the method reduces the rubidium impurity in the final cesium sample to less than 2 parts per thousand. This separation is accomplished by means of an ion exchange step employing sodium hydroxide elution from a phenolic methylene sulfonic-type resin, followed by one or more precipitation steps for final purification of the cesium from sodium and potassium.

CLASSICAL precipitation methods were not found satisfactory for the isolation and purification of small amounts of cesium from large excesses of the other alkali metals. These methods involve separation of the heavy alkali group—potassium, rubidium, and cesium—from sodium, provided sodium is not present in overwhelming abundance, followed by separation of cesium, rubidium, and potassium from each other. Cesium and rubidium are especially difficult to separate, although some success has been achieved with paper chromatography (*8*) and solvent extraction (*10*). In the past few years great success has also been achieved by chromatographic elution of the alkalies with hydrochloric acid from cation exchange resins (*1, 2, 4, 6, 7*). However, the ion exchange methods involve approximately equal amounts of the alkalies in the starting product for a good separation of cesium from rubidium. Any large excess of one or more of the alkalies would involve the use of large equipment and long elution times, because the procedures require adsorption of the entire starting product on the exchange medium and consequent separation from that point. These requirements become prohibitive in the case of separation of a few milligrams of cesium from a pound of other alkali metals.

Miller and Kline (*9*) have reported an exceptional ability of Amberlite IR-100 cation resin to adsorb cesium out of a concentrated sodium solution at pH 13, as well as good retention of cesium when this same solution is eluted through a column of the resin. The special selectivity for cesium may be ascribed to the phenolic structure of the resin, because the nuclear sulfonic resins under the same conditions do not show this property.

The following experiments describe the conditions for adsorption of cesium from a highly concentrated alkali salt solution onto a phenolic methylene sulfonic resin column, as well as the method for the removal of rubidium and the bulk of the potassium by eluting with a 0.5N sodium hydroxide solution.

EXPERIMENTAL

Because the similar Amberlite types are no longer being manufactured, phenolic methylene sulfonic resin, Duolite C-3 (Chemical Process Co., Redwood City, Calif.), was used in these experiments. Duolite C-3 is produced in a single coarse grade, two thirds of which is 20 to 50 mesh and the remainder larger than 20 mesh. In all the experiments except one the resin was used unaltered. After exploratory runs had been made with radioactive cesium and rubidium tracer to determine roughly the conditions by which cesium would be adsorbed on the resin column and rubidium would be eluted, the experimental runs were made without tracer and the samples analyzed for alkali content by a flame photometric method (*5*).

In each experiment the cesium and rubidium were contained initially in from 1 to 2 liters of saturated sodium chloride solution (roughly 1 pound of salt). This solution was made 0.5N in hydroxide ion with sodium hydroxide pellets and added to a resin column 1.2 cm. in diameter and either 80 or 120 cm. long. The resin was then eluted with reagent grade 0.5N sodium hydroxide

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solution to remove rubidium. The cesium was finally stripped from the resin column with 6*N* hydrochloric acid. Table I gives the conditions and results of the experiments.

Column 2, Table I, gives the length of resin bed measured before the run. Shrinkage is considerable when the bed is eluted with the strong acid. The flow rates were held constant for the adsorption, elution, and stripping step for each run. The amount of cesium recovered at the end of the run (weight independent of chloride) is given in column 6. The final cesium product is made up from both the cesium added and that contained in the sodium chloride as a minute impurity. Because the cesium impurity is unknown. no absolute statement can be made as to the recovery of cesium in the procedure. However, the final samples always contained more cesium than was added; thus, the procedure appears to be quantitative. The rubidium-cesium ratios given in the table are based on the amount of cesium obtained in the product. The decontamination or reduction in rubidium (column 9) is a ratio of the relative amount present initially to that present finally.

Table I. Ion Exchange Separation of Cesium from Rubidium

Run	Column Length, Cm.	Flow Rate, Ml. per Minute	Volume 0.5*N* NaOH, Liters	Volume 6*N* HCl, Liters	Cesium, Mg.	Ratio, Rb/Cs Initial	Ratio, Rb/Cs Final	Reduction Factor
1	80	35	3.0	5.0	8	4.4	0.12	36
2	80	35	6.0	6.0	9	1.7	0.10	17
3	80	35	10.0	6.0	13	3.5	0.06	60
4	117	7	15.0	6.0	18	8.6	0.02	400
5[a]	119	7	12.5	5.0	37	5.4	0.006	900
6	117	2	12.5	3.0	36	5.4	0.0016	3400

[a] Ground resin

DISCUSSION

Comparison of the various runs indicates that the rubidium reduction is very sensitive to the flow rate—more so than to the amount of eluent used at a given flow rate. In order to test the effect of smaller resin particle size, the resin used in run 5 was ground so that two thirds of it was 20 to 50 mesh, with the remainder from 50 to 200 mesh. This produced a better separation, which can be seen by comparing runs 4 and 5, where the conditions were otherwise essentially the same.

By checking the sodium hydroxide eluate for rubidium it was found that the bulk of the rubidium comes off in the first few hundred milliliters; thereafter the amount being removed from the column diminishes very slowly, giving a long tail over several liters. This fact, combined with the enhanced separation obtained by using smaller resin particles and slower flow rates, leads to the conclusion that the rate of diffusion of the rubidium through the resin particles is the controlling factor for optimum separation under given conditions.

In addition to the cesium and small amounts of rubidium, the hydrochloric acid stripping solution also contained the sodium which was still adsorbed on the column at the end of the sodium hydroxide elution step. Because the sodium hydroxide reagent from which the eluent is made contains a potassium impurity (0.1%), potassium is also present in the hydrochloric acid strip. For a column 117 cm. long and 1.2 cm. in diameter the potassium contamination was approximately a constant, 3 mg. This amounts to 8% of the weight of cesium present in runs 5 and 6.

To reduce the stripping solution to a form suitable for analysis by flame photometry, the hydrochloric acid and excess sodium had to be removed. The solution was evaporated almost to dryness, the salts were taken up in water, and then the solution was made slightly basic with sodium hydroxide. Two grams of sodium tetraphenyl boron, previously dissolved in water, were added to the solution to precipitate cesium, rubidium, and potassium (*12*). The precipitate was filtered and fumed with nitric and perchloric acids to destroy the organic matter. This procedure reduced the sodium to 40 to 70% of the total weight of salts present.

To test the effectiveness of two successive ion exchange steps, run 4 was recycled after being analyzed. The second column was 10 mm. in diameter and 20 cm. long, the flow rate was 0.5 ml. per minute, the eluent volume was 1 liter, and the stripping volume was 500 ml. Analysis of the product gave a final rubidium to cesium ratio of 0.0034 and a potassium contamination of 0.4%. This amounts to a rubidium reduction factor of 6 for the small column alone and 2400 for both columns. The potassium is reduced because the eluent holdup is smaller in the small column. The sample was precipitated from the evaporated stripping solution as the chloroplatinate instead of the tetraphenyl boron compound. This chloroplatinate gave a final sodium contamination of 1.7%.

In cases where the potassium and sodium content of the final sample must be reduced below 1%, an extra precipitation can be used. Several methods are known for removing potassium and sodium from cesium. (However, these methods are poor for removal of rubidium.) Two possible methods are precipitation of cesium bismuth iodide (*3*) or cesium silicowolframate (*11*). The silicowolframate method used in these experiments gave a final potassium and sodium content of less than 0.5% each. The cesium may be precipitated finally as the chloroplatinate or perchlorate for gravimetric assay if desired.

Duolite C-3 cation exchange resin was obtained in the hydrogen form. Some runs were made directly on the resin without pretreatment and some after conversion to the sodium form with sodium hydroxide solution. Neither form can be said to have an advantage because it requires only a few milliliters of concentrated sodium chloride test solution to saturate the resin completely. However, the resin does contain iron which is eluted from the column during the stripping step. The iron can be removed before the run by washing the column with hydrochloric acid. The presence of iron does not interfere with the cesium-rubidium separation, but it must be removed if the treatment of the stripping solution involves addition of a base.

ACKNOWLEDGMENT

The author gratefully acknowledges the contribution of Minoru Honma of this laboratory, who ran the flame photometric analyses on the samples.

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RECEIVED for review October 31, 1955. Accepted March 26, 1956.

Spectrophotometric Assay and Identification of Monosaccharides

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Accurate and rapid assay of monosaccharides is made possible by the highly reproducible rate of periodate oxidation determined spectrophotometrically. Furthermore, the fact that most of the monosaccharides possess distinctive rates of periodate oxidation permits their simultaneous identification and assay. The method is also applicable to eluates of paper chromatograms.

RECENT work on the characterization of the polysaccharide associated with influenza virus (*3*) required an assay with a sensitivity in the 1 to 10 γ range for each of the constituent monosaccharides separated by paper chromatography. The majority of the conventional microanalyses for sugars were found to have lower limits of sensitivity somewhat above this range. Prominent among these is the periodate method of Flood and Hirst (*2*), in which a determination is made of the formic acid produced when the sugars are oxidized with sodium periodate for 20 minutes at 100° C.

Dixon and Lipkin (*1*) recently reported a method for the quantitative determination of vicinal glycols based upon the spectrophotometric measurement of periodate utilization at 220 mμ, the absorption maximum of periodate. It was apparent that this method, if it could be applied to the analyses of monosaccharides, would possess not only the requisite sensitivity and accuracy but also ease and speed.

The initial results were disappointing, in that periodate oxidation of the sugars under the conditions of the method of Dixon and Lipkin is slow, indeed requiring days at room temperature. The application of heat, while greatly speeding up the reaction, led under no circumstances to the calculated utilization of periodate. This is understandable in terms of the many subtle side reactions of periodate which could occur in such a system.

There was, however, remarkable reproducibility in the curves obtained by plotting periodate consumption (in terms of the absorbance of the reaction mixture) against the initial concentration of a given monosaccharide under constant conditions of time and temperature. Not only was excellent agreement obtained in replicate determinations, but also analyses made on different days agreed exceptionally well. It thus appeared possible to obtain highly accurate assays of monosaccharides by reference to standard curves in which the consumption of periodate during an arbitrary time interval was plotted against concentration of sugar. Furthermore, the fact that such curves showed characteristically distinct slopes for different monosaccharides suggested that this might be a supplementary method for the qualitative identification of sugars.

The lack of stoichiometry encountered in the attempt to modify the procedure of Dixon and Lipkin is explained and resolved by the recent spectrophotometric studies by Marinetti and Rouser (*4*) of the periodate oxidation of ribose-5-phosphate. By employing the spectral range of 280 to 310 mμ, which permitted the use of buffers, these workers were able to obtain a stoichiometric consumption of periodate with several sugar phosphates and with glucose. However, the method presented here appears to offer the advantages of greater simplicity, shorter assay period (3 hours rather than 23), and a considerably greater sensitivity by virtue of using the absorption maximum of periodate at 220 mμ. On the other hand, limitations are placed on the method by the fact that phosphate and acetate buffers, as well as other inorganic substances and organic materials, absorb in this region.

APPARATUS

Beckman spectrophotometer, Model DU. Quartz cells (standard 3-ml. size) of 1-cm. light path.

The assays are carried out in ordinary 30-ml. test tubes which are thoroughly cleaned by immersion in chromic acid cleaning solution, followed by adequate rinsing with tap and distilled water.

Rubber stoppers may be used, provided they are not in intimate contact with the contents of the assay tube.

REAGENTS

Sodium Metaperiodate, $10^{-4}M$. This solution can conveniently be prepared by simply dissolving 21.4 mg. of anhydrous sodium metaperiodate in 1 liter of water. A solution containing 5 ml. of the fresh periodate solution plus 1 ml. of water is commonly found to have an absorbance reading of ca. 0.804 at 227 mμ. However, in a matter of 24 hours this drops to about 0.795, a plateau value which remains constant for about a week. Fresh periodate solutions are allowed to age for 24 hours and the standard curves are based on the latter absorbance value.

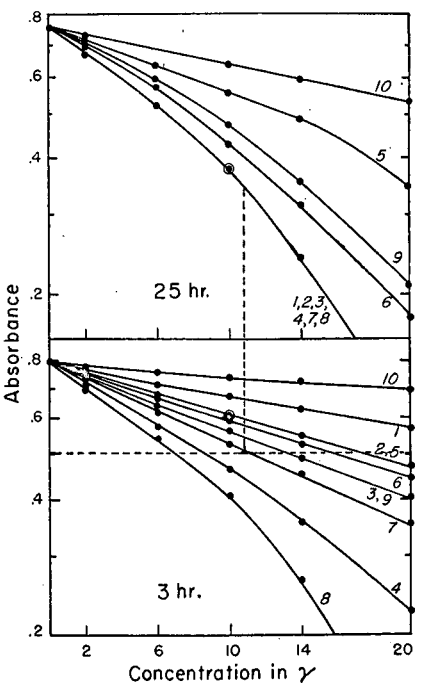

Figure 1. Periodate utilization curves (absorbance *vs.* concentration) of several monosaccharides

1. D-Glucose
2. D-Galactose
3. D-Mannose
4. D-Ribose
5. D-2-Deoxyribose
6. L-Fucose
7. L-Sorbose
8. D-Sorbitol
9. D-Fructose
10. D-Glucosamine

Standard Monosaccharide Solutions (20 γ per ml.). One hundred milligrams of the carefully dried monosaccharide is made up to 10 ml. in a volumetric flask; 1 ml. of this solution is then diluted to 500 ml. The following sugars were used: D-glucose, Merck, C.P.; D-galactose, D-mannose, and D-ribose, Nutritional Biochemical Corp., D-2-deoxyribose, California Foundation for Biochemical Research, C.F.P.; L-fucose, Mann Research Laboratories, C.P.; L-sorbose, recrystallized from a

commercial source; and D-sorbitol, D-fructose, and D-glucosamine, Pfanstiehl Laboratories, C.P. All the sugars were chromatographically pure.

QUANTITATIVE PROCEDURE

One milliliter of the solution containing the standard or the unknown (0 to 20 γ) is pipetted into 5 ml. of $10^{-4}M$ sodium periodate solution. In the case of standard curve determinations or assays of solutions containing only one pure sugar, the zero-hour absorbance reading may be assumed to be that of the periodate blanks (5 ml. of $10^{-4}M$ periodate and 1 ml. of water) which are run concurrently. However, when other organic substances, which may contribute to the absorbance of the assay mixture, are suspected to be present, it is necessary to determine the absorbance immediately after addition of sample to the periodate solution. This is particularly true of chromatographic eluates.

Absorbance readings are taken at 227 mμ (slit width, 0.8 to 1.2 mm.) at 3 hours. The contents of the assay tubes are simply poured into a cuvette which has been standardized against distilled water.

In this manner the standard curves of absorbance *vs.* concentration of a series of ten important monosaccharides were determined (Figure 1). The logarithmic scale of the ordinate in Figure 1 was used in order to demonstrate certain characteristics of the curves. Of greatest interest is the linearity of the 3-hour period in the range of 1 to 10 γ for certain sugars—e.g., ribose—and 1 to 20 γ for other sugars—e.g., glucose.

Many of the data in Figure 1 are drawn from duplicate runs, each at the 2-, 6-, 10-, 14-, and 20-γ levels, on three separate days at room temperature (about 24° C.) and daylight illumination. The range of absorbance readings seldom deviates from the mean by more than ± 0.004 absorbance unit at the 3-hour period. It is obvious from these data that an accuracy in excess of 98% can be expected at this time period. The range, however, becomes somewhat broader at the 24- and 48-hour periods, which are more susceptible to temperature fluctuation than the 3-hour period.

Four disaccharides—maltose, cellobiose, lactose, and sucrose—have also been found to possess distinctive and highly reproducible periodate oxidation curves under the same conditions.

Under room conditions marked by drastic daily fluctuations of temperature, it would be necessary to reinvestigate the constancy of the standard curves.

QUALITATIVE IDENTIFICATION

The approach to this problem will depend upon whether or not the exact concentration of the monosaccharide is known.

An unknown monosaccharide assayed at a known concentration within the conditions of the general procedure will yield an absorbance value which will fall upon its standard curve at that concentration. It will be necessary to obtain absorbance readings at both the 3-hour and 25-hour periods in order to permit differentiation of the 3-hour "family" curves. Of the ten monosaccharides shown in Figure 1, all the sugars having the same periodate oxidation curve at the 3-hour period possess different curves at the 25-hour period.

The convergence of the 3-hour curves at low concentration dictates a lower limit of about 4 γ for this method. The upper limit is governed by the upper region of the standard curves. Many of the sugars—e.g., glucose—can be analyzed at concentrations as high as 20 to 30 γ; however, this great excess of other sugars—e.g., ribose—would use up the periodate before the 3-hour assay period.

The following example illustrates the identification of a monosaccharide at known concentration. One milliliter of a solution containing 10 γ of the unknown monosaccharide yielded an absorbance reading of 0.609 at the 3-hour period and 0.375 at the 25-hour period. These absorbance values at the 10-γ level are shown marked with small circles in Figure 1. The 3-hour reading at the 10-γ concentration falls on the galactose-deoxyribose curve, while the 25-hour reading coincides only with the galactose standard curve.

The use of both 3-hour and 25-hour absorbance values allows not only differentiation of "family" curves but also simultaneous determination of the concentration and identity of an unknown monosaccharide. In all cases where the concentration is unknown, the following procedure is recommended. The solution containing the unknown sugar is either diluted or concentrated and run at several concentrations according to the general procedure. The dilution having a 3-hour absorbance reading of 0.50 ± 0.02 is read again at 25 hours. The horizontal dotted line through the absorbance level of 0.50 at the 3-hour period in Figure 1 bisects all of the standard curves, with the exception of glucosamine and glucose (these are included by extension of the graph). The choice of this absorbance value thus ensures a concentration within the limits of the method.

The absorbance value at the 25-hour period is marked on each of the 25-hour curves and the concentrations corresponding to each of the intersections with this absorbance value are referred to the 3-hour curve. The format of Figure 1 permits easy reference of concentrations between the 3-hour and 25-hour curves by simply laying a rule upon the diagram. The unknown will be identical to that standard upon whose curve of absorbance *vs.* concentration both the 3-hour and 25-hour assay absorbance readings will fall at the same concentration.

For example, 1 ml. of an unknown is found to yield an absorbance reading of 0.503 at the 3-hour period and 0.342 at the 25-hour period. Only sugar 7, L-sorbose, will satisfy the requirement (see vertical dotted line of Figure 1) of possessing these two absorbance values at the same concentration. At the same time its concentration has been fixed at 11.2 γ.

An analysis of Figure 1 will reveal that at the concentrations of mannose and fucose (Nos. 3 and 6) which yield 0.500 absorbance unit at the 3-hour period there is also an equivalence in the absorbance values for the two sugars at the 25-hour period. Therefore, this method cannot distinguish between mannose and fucose, although at another absorbance level at the 3-hour period they can be differentiated.

The assay should be made in triplicate and the precision must be in the order of $\pm$ 0.004 absorbance unit at the 3-hour period, in order to make a differentiation of curves which fall close together.

This method is particularly valuable in the case of a chromatographic eluate where another parameter of identification would be useful in addition to a quantitative assay of the monosaccharide.

The method is also applicable to several disaccharides. It shows promise as a rapid method of differentiating glycosyl isomers—e.g., maltose and cellobiose.

APPLICATION TO PAPER CHROMATOGRAPHIC ANALYSIS

Two potentially disturbing factors affect the analyses when eluates of paper chromatograms are to be assayed. The first of these is the contribution to the absorbance of lingering chromatographic solvents and oxidative decomposition products of lignin present in many types of filter paper. Solvent contribution may be reduced to the vanishing point by steaming the chromatogram in an autoclave for 10 minutes at 90° to 100° C. before elution of the sugars. Care should be taken at this point that the sugars are not affected. In most cases, the absorbance due to organic substances derived from the paper is very small and variable; however, for the sake of accuracy it should be measured as the difference between the periodate blank and the zero-hour reading of the assay.

A second source of interference may come from periodate-oxidizable substances extracted from the paper. This effect is corrected for by eluting and assaying, in the same manner as the

Table I. Recovery of Ribose and Glucose from Paper by Periodate Method

Preparation	Amt., γ	Absorbance Readings 0 hr.	Absorbance Readings 3 hr.	Correction Factor[a]	Corrected Assay Absorbance	Calcd. Absorbance Standard Curve	Recovery, %
Ribose	4.0	0.810	0.660	−0.003	0.657	0.650	98
	10.0	0.801	0.464	+0.006	0.470	0.467	99
Glucose	2.5	0.790	0.740	+0.017	0.757	0.758	100
	5.0	0.796	0.713	+0.011	0.724	0.728	101
Paper blanks		0.811	0.799				
Periodate blank		0.795	0.795				

[a] Correction factor. ± Difference in absorbance reading of paper blank at 0 and 3 hours. ± Difference in absorbance reading of 0-hour assay and 0-hour periodate blank (see text).

unknowns and standards, blank sections of paper of the same size and from the same chromatogram for which the sugar spots were cut. This correction, expressed as the difference in absorbance between the paper blank at zero-hour and assay-hour period, is added to the readings at the various time intervals.

The treatment of typical data is illustrated in Table I.

In these runs known amounts of the monosaccharides were spotted by micropipet on a sheet of Whatman No. 1 paper which had been run two-dimensionally in collidine: HOH (saturated) and BuOH:HAc:HOH (4:1:5) and dried. The paper was freed of lingering solvents by the steaming procedure previously described. The areas corresponding to the deposits of sugars were cut out, folded, and immersed in 10 ml. of water for 4 hours with continued shaking at 37° C. This method of elution has been found fully effective and convenient. The eluate was centrifuged in order to remove paper lint which might otherwise interfere with the absorbance reading. One milliliter of the eluate was then assayed according to the general quantitative procedure, yielding the data in Table I. Paper blanks and periodate blanks were run simultaneously. At least one level of standard is always run, in order to be certain that standard conditions are prevailing.

ACKNOWLEDGMENT

The author wishes to acknowledge gratefully the many helpful suggestions of C. A. Knight, E. W. Putman, and D. L. MacDonald, all of this university. This investigation was supported in part by a research grant, RG-4559, to C. A. Knight from the National Institutes of Health, Public Health Service, and by grants from the Lederle Laboratories Division, American Cyanamid Co., and the Rockefeller Foundation.

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RECEIVED for review December 27, 1955. Accepted April 13, 1956

Determination of Traces of Fatty Amines in Water

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A method has been developed for determining traces of high molecular weight fatty amines in water. The procedure should be applicable to the control of amine concentration in steam condensate systems where fatty amines are added to inhibit corrosion. Amine concentration is determined by titrating with an anionic surface active agent to the disappearance of pink color due to an amine-eosin complex. A calibration curve is given for the concentration range of 0.5 to 10 p.p.m.

FATTY amines of high molecular weight have been used successfully for some time in steam condensate systems to inhibit corrosion. The efficient control of this treatment requires a constant check on the concentration of amine in the condensate waters at the 1 to 10 p.p.m. level. Therefore, a direct, rapid test for determining the concentration of fatty amine in water at these low levels is desirable. This paper describes such a test, which should be applicable to steam condensates.

Bouilloux (*2*) found that very dilute solutions of fluorescein, or its derivatives, in organic solvents are colorless, but form colors upon the addition of certain amines. Eosin, in particular, was very sensitive, forming a pink color in the presence of very small quantities of amine. He attributed the pink color to a quinoid-type structure resulting from the formation of an eosin-amine salt. Prudhomme (*4*) used this color formation of amine with eosin to determine quinine in urine. A buffered sample solution containing eosin was extracted with chloroform and the chloroform extract containing the colored quinine-eosin salt was compared with the color of standard solutions.

Harper, Elliker, and Mosely (*3*) utilized the red color resulting from the reaction of eosin and quaternary ammonium salts in a quantitative titration procedure for determining the latter at the 10 to 300 p.p.m. level. The titration was carried out with an anionic surface active agent which replaced the eosin in the quaternary-eosin salt and destroyed the color.

The procedure described below is essentially that used by Harper, Elliker, and Mosely (*3*) for quaternary ammonium salts. A buffered sample of water containing fatty amine is shaken up with a dilute solution of eosin in tetrachloroethane. The amine forms a pink tetrachloroethane-soluble compound with eosin. The resulting mixture is then titrated with a solution of sodium lauryl sulfate, previously calibrated against known quantities of amine, until the pink color in the nonaqueous layer has disappeared. Analyses by this method of known mixtures containing fatty amine in water in the range of 0.5 to 10 p.p.m. indicate an accuracy within 0.5 p.p.m. of amine.

REAGENTS AND APPARATUS

Indicator Solution. Dissolve 10 mg. of Eosin yellowish (sodium salt of tetrabromofluorescein) in 100 ml. of analytical reagent grade acetone. Add 10 ml. of the acetone solution to 90 ml. of tetrachloroethane. Remove the reddish color from the tetrachloroethane solution by adding 0.5 gram of citric acid and shaking for 1 minute. Filter through Whatman No. 1 (or equivalent grade) filter paper.

Buffer Solution. Prepare a 5% aqueous solution of citric acid and adjust to pH 3.5 with 0.1*N* sodium hydroxide. Add 1% tetrachloroethane to prevent mold growth.

Anionic Surface Active Agent Solution. Prepare a 0.01% aqueous solution of sodium lauryl sulfate. This solution should be recalibrated frequently and discarded when deterioration becomes evident.

Test tubes, $^1/_2 \times 5$ inches, are rinsed with alcohol and acetone and dried before use.

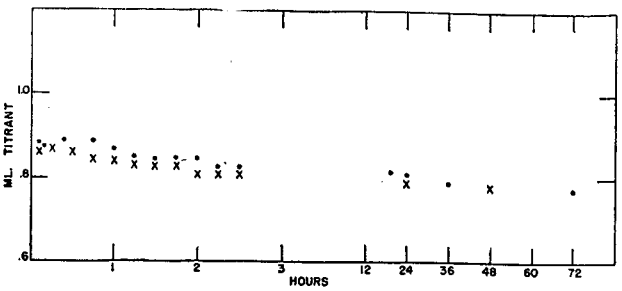

Figure 1. Amine adsorption in coated flasks

● Hydrogenated tallow amine
× Octadecylamine

One-Liter Volumetric Flasks. Coat inside of flasks with Beckman Desicote (*1*) to cut down adsorption of amine on the flask surfaces.

Buret, 10-ml. capacity.

PROCEDURE

Standardization. Prepare a series of standard aqueous amine solutions covering the desired concentration range in the following manner. Weigh the required amount of amine (or equivalent amount of amine acetate) into a 5-ml. beaker. Dissolve the amine in about 1 ml. of isopropyl alcohol and pour into a 1-liter volumetric flask containing approximately 900 ml. of water. Wash out the beaker into the flask with a total of 9 ml. of isopropyl alcohol. In this transfer do not allow the amine to touch the inside of the flask neck, but pour directly into the water, using a stirring rod. Swirl the flask to disperse the amine, then make up to volume with water and shake. For very low concentrations (0.5 to 3 p.p.m.) dilute aliquots from a 10 p.p.m. stock solution in a 1-liter flask.

Titrate a 5-ml. aliquot of each standard solution as described below within 1 hour of preparation. Prepare a calibration curve by plotting milliliters of anionic solution against parts per million of amine.

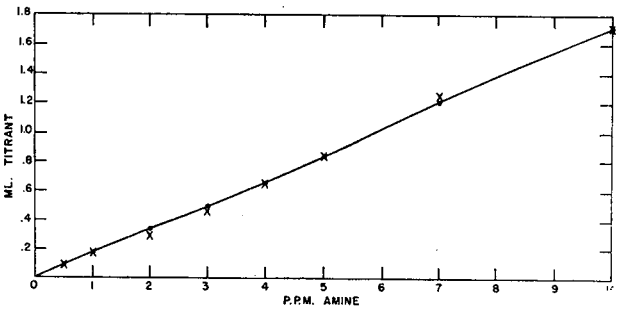

Figure 2. Calibration curve

● Hydrogenated tallow amine
× Octadecylamine

Determination. Fill a 1-liter volumetric flask with the sample solution. Within 1 hour, rinse a 5-ml. volumetric pipet three times with the sample solution in the flask, discarding the washings; then pipet 5 ml. of the same sample solution into a test tube. Add 1 ml. of the indicator solution, followed by 0.1 ml. of the buffer solution. Place thumb over the test tube mouth, shake vigorously for approximately 30 seconds, and allow the two liquid layers to settle. A pink color in the bottom layer indicates the presence of amine. A polyethylene thumb covering was found helpful in protecting the thumb.

Titrate with the anionic solution to the disappearance of the pink color in the lower liquid layer. During the titration, shake the test tube between additions of each 0.05 ml. of the anionic solution, allowing the bottom layer to settle partly, and comparing the color of the bottom layer against a white background with that of a blank which contains 5 ml. of water and the prescribed volumes of indicator and buffer solutions and which is shaken simultaneously. Approximately 0.1 ml. before the end point there is a noticeable increase in the rate of settling of the two layers. This rate of settling decreases on the addition of more titrant.

Table I. Adsorption of Amine on Polyethylene

Amine	Immediately	2 Hr.	4 Days	5 Days	7 Days	11 Days	13 Days	24 Days
H26D[a], p.p.m.	10	8.8	3.7	2.8	2.2	1.2	1.0	0.3
7D[b], p.p.m.	10	9.2	5.9	5.1	4.1	2.7	2.3	0.5

[a] Distilled hydrogenated tallow amine.
[b] Distilled octadecylamine.

DISCUSSION

A number of compounds which might be present in small amounts in technical grade, primary fatty amines were tested for color formation in this procedure. Dioctadecyl and trioctadecyl amines also gave a pink color, stearamide gave a very faint pink color in concentrations greater than 30 p.p.m., whereas stearonitrile, *N*-octadecylacetamide, and stearic acid gave no color even in very large concentrations. Ammonia in larger than 30 p.p.m. concentrations gave pink color only in the upper aqueous layer. Tap water gave no color and behaved like distilled water when primary amine was added. Several samples of steam condensate water with added amine behaved normally when checked by this procedure.

Considerable difficulty was encountered in the early part of this work because of the adsorption of fatty amines from water onto surfaces of glass and polyethylene containers. Standard aqueous solutions containing amine in the parts per million range gave appreciably lower titrations on standing, amines of slightly different molecular weight or extent of unsaturation gave appreciably different titrations, amine acetates gave titrations different from equivalent amounts of amine, and transferring amine solutions from one container to another resulted in significantly lower titrations. This loss of amine by adsorption onto the container surface was appreciable in glass and prohibitive in polyethylene.

Table I shows the extent to which amine was lost by adsorption when 10 p.p.m. aqueous solutions of a hydrogenated tallow and an octadecylamine were stored in polyethylene bottles. However, this undesirable adsorption could be cut down appreciably by coating the inside of glass flasks with a silicone such as Beckman Desicote.

When aqueous solutions containing equivalent amounts of amine were prepared in flasks coated with Desicote, there was no significant difference in titration with tallow, hydrogenated tallow, or octadecylamine. The corresponding acetates at equivalent amine concentration also gave the same titrations. Figure 1 shows the extent of adsorption from 5 p.p.m. aqueous amine solutions in flasks coated with Desicote. It is evident that, if coated flasks are used for both sample and standard solutions and if titrations are carried out within 1 hour, adsorption losses are negligible.

Figure 2, a calibration curve obtained with octadecylamine and a hydrogenated tallow amine, shows the linear relationship between amine concentration and volume of titrant. The corresponding acetates at the 5 p.p.m. amine level gave titrations within 0.4 p.p.m. of those obtained with the amines.

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RECEIVED for review January 30, 1956. Accepted April 12, 1956. Paper 190, Journal Series, General Mills, Inc.

Chemiluminescent Indicator Titration of Lead with Potassium Chromate

In Lead-Tin Alloys and in Metallic Samples Containing Lead, Tin, Antimony, and Arsenic

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Lead in lead-tin alloys and mixtures was rapidly determined by titrating with potassium chromate. Siloxene indicator was used. Interference by tin, antimony, and arsenic was avoided by elimination of these elements as bromides prior to titration.

LEAD has been determined (*5*) with an accuracy of 0.7 part per 1000, and an average deviation of 1.3 parts per 1000, by titrating it with potassium chromate in a dark chamber titrator (*4*) and employing siloxene chemiluminescent indicator (*3*). The light emitted at the end point was detected by a Photovolt multiplier photometer 520-A (*6*). The pH at the beginning of the titration was approximately 2.6.

To study the possibility of interference by other metals, the present authors (*5*) titrated solutions which were 0.1*M* with respect to an additional metal ion as well as 0.1*M* with respect to lead. The additional metal ions were manganese (II), nickel (II), iron (III), zinc (II), calcium (II), and magnesium (II). These ions do not form a precipitate with chromate at the pH employed. The errors encountered were for the most part between 1 and 2 parts per 1000. The largest error, which was encountered with the zinc solution, was 3 parts per 1000.

To determine the effect of the presence of tin, lead was titrated in a solution of an alloy containing 47.83% tin and 52.17% lead. The results, which were totally unsatisfactory, were low to the extent of 96 parts per 1000 and had an average deviation of 13 parts per 1000. In the unsatisfactory method used the alloy was dissolved in 6*M* nitric acid, after which the pH was adjusted to 2.6 with ammonia. Most of the tin was present as hydrated stannic oxide. If the effect of the stannic oxide was merely to absorb the light evolved by the indicator, high results would have been produced, because additional chromate would be required to attain a stronger light. The low results can be accounted for if it is assumed that stannic oxide adsorbs lead ions or that some stable complex ion of lead is formed; the end point, therefore, comes too soon.

In order to eliminate the interference produced by tin, attempts were made to drive off the tin as stannic iodide by treatment with ammonium iodide. The results were completely unsatisfactory. The decision to use hydrobromic acid (*1, 2*) instead of ammonium iodide, however, led to the development of the method described in this paper.

The lead-tin alloy, finely divided and containing about 0.5 gram of lead, is treated with 5 ml. of 48% hydrobromic acid in a covered 100-ml. beaker. Then 5 ml. of bromine reagent, previously prepared from 2 volumes of bromine and 7 volumes of 48% hydrobromic acid, is added dropwise to the covered sample in a good hood. The watch glass and sides of the beaker are washed with 48% hydrobromic acid from a small dropping bottle, and the watch glass is discarded. The solution is then evaporated to dryness in the hood with the use of an overhead radiant heater to avoid spattering; 45 minutes to 1 hour is required for the operation. The heating is continued for 15 minutes after dryness is reached. This operation eliminates tin as stannic bromide. The beaker is cooled and the sides of the beaker are washed down with about 8 ml. of 6*M* nitric acid. The solid residue is broken up with a stirring rod and the beaker containing the stirring rod is covered and placed on a hot plate in the hood until the color of bromine is removed completely. The sides of the beaker are then washed with water. The volume is kept below 25 ml. The pH is adjusted to 2.6 to 2.8 by the addition of ammonia. A glass electrode pH meter is employed. The stirring rod is then washed off and discarded. The solution is now ready for titration.

Eight samples can be treated by this procedure in less than 2 hours.

In titrating the samples in the dark chamber titrator, approximately 75 mg. of siloxene indicator was used and the solution was stirred mechanically. The titrations reported in Tables I and II were continued until the pointer of the photometer, when operating on the No. 3 scale, moved swiftly from zero to one unit, which corresponds to 0.002 microlumen. The samples of Table III were titrated to a predetermined end point.

The equation for the reaction involved in the titration is:

$$Pb^{++} + CrO_4^{--} \rightarrow PbCrO_4$$

Two lead-tin alloys were prepared from chemically pure lead and chemically pure tin. The temperature was very carefully controlled, so as to be only slightly above the melting point, and the melt in a borosilicate glass container, stirred with a glass rod, was held in the furnace only long enough to ensure complete mixing. No smoke or oxide film was observable and the melt had a mirrorlike surface. Each alloy was disintegrated by filing and was carefully sampled. Because of the great care exercised, it was felt that the errors in preparing these alloys were exceedingly small. The compositions were 52.17% lead and 47.83% tin, and 70.83% lead and 29.17% tin, respectively.

Table I. Titration of Lead in 52.17% Lead–47.83% Tin Alloy with 0.1000*M* Potassium Chromate

(75 mg. of siloxene indicator)

Sample, G.	Lead in Sample, G.	Potassium Chromate, Ml.	Lead Found by Analysis, G.	Lead, %	Deviation, Parts/1000
1.1912	0.6214	30.00	0.6216	52.18	0.76
1.1913	0.6215	30.02	0.6220	52.21	0.19
1.1920	0.6219	30.05	0.6226	52.23	0 19
1.1923	0.6220	30.02	0.6220	52.17	0.95
1.1921	0.6219	30.10	0.6237	52.32	1.91
				Mean 52.22	0.80

Table I gives the results of the titration of the 52.17% lead–47.83% tin alloy. The precision obtained, in terms of the standard deviation, is 1.1 and in terms of average deviation is 0.8. The mean error involved is 1.0 part per 1000.

Table II gives the results of the titration of the 70.83% lead–29.17% tin alloy. The precision obtained, in terms of the standard deviation, was 2.5 parts per 1000 and in terms of the average deviation was 1.8. The error was 3.3 parts per 1000.

The small average error of 1.0 part per 1000 presented in Table

Table II. Titration of Lead in 70.83% Lead–29.17% Tin Alloy with 0.1000*M* Potassium Chromate

(75 mg. of siloxene indicator)

Sample, G.	Lead in Sample, G.	Potassium Chromate, Ml.	Lead Found by Analysis, G.	Lead, %	Deviation, Parts/1000
0.8765	0.6208	29.85	0.6185	70.56	0.53
0.8770	0.6212	29.80	0.6175	70.41	2.50
0.8782	0.6220	29.90	0.6195	70.54	0.79
0.8769	0.6211	30.00	0.6216	70.85	3.29
				Mean 70.60	1.78

Table III. Titration of Lead in Samples Containing Antimony, Arsenic, and Tin with 0.1000*M* Potassium Chromate

(75 mg. of siloxene indicator)

Sample No.	Lead in Sample, G.	Potassium Chromate, Ml.	Lead Found by Analysis, G.	Error, Parts/1000
1	0.6229	30.02	0.6220	1.44
2	0.6230	29.97	0.6210	3.21
3	0.6216	30.05	0.6226	1.60
4	0.6238	30.10	0.6237	0.16
5	0.6226	30.10	0.6237	1.77
			Mean error	1.64

Table IV. Standardization of 75 Mg. of Siloxene Indicator in Titration of 30.00 Ml. of 0.1000*M* Lead Nitrate with 30.00 Ml. of 0.1000*M* Potassium Chromate

Titration	Photometer Reading: Scale divisions	Photometer Reading: Microlumen
1	1.0	0.0020
2	1.5	0.0030
3	2.0	0.0040
4	2.0	0.0040
Mean	1.6	0.0032

I as compared with the corresponding value of 3.3 parts per 1000 obtained from Table II probably results from a fortuitous balancing of errors, as the errors involved in the physical measurements alone would lead one to expect an accuracy within 2 parts per 1000. Errors of method could, of course, increase this to an even larger value.

Eight samples of alloy were subjected to the entire analysis, including pH adjustment and titration, in 2.5 hours.

Based on the properties of antimony and arsenic and their bromides (*1*, *2*), it appeared probable that the treatment employed for the elimination of tin would also eliminate both antimony and arsenic. Consequently, five mixtures of chemically pure portions of lead, antimony, arsenic, and tin were prepared. These mixtures had the compositions:

Sample	1	2	3	4	5
Lead, %	69.19	69.08	69.11	69.17	69.00
Antimony, %	2.42	2.27	2.20	2.27	2.43
Arsenic, %	0.69	0.79	0.53	0.60	0.72
Tin, %	27.70	27.86	28.16	27.96	27.85

These samples were dissolved and prepared for titration in exactly the same manner as the two lead-tin alloys previously considered. Table III gives the results of the titrations.

For very accurate work standardization of the indicator is desirable. Variations in the method of preparing the siloxene indicator and its age, which can conceivably affect the end point, can be taken into account by standardization. Thus for the titrations presented in Table III the indicator had been previously standardized by titrating 30.00 ml. of 0.1000*M* lead nitrate solution with 30.00 ml. of 0.1000*M* potassium chromate (Table IV).

As a consequence, the titration of the samples shown in Table III was continued until the photometer reading was 1.6 on the No. 3 scale.

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RECEIVED for review November 1, 1955. Accepted April 6, 1956.

Dielectric Values for the System Water—Ethyl Acetate

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A study has been made of the relationship between a varying moisture content of ethyl acetate and the corresponding dielectric values, water being regarded as the solute. Up to nearly 2% of water, by weight, this correlation is linear. Conspicuous departure from linearity occurs only when sufficient time has elapsed for hydrolysis to intervene, a matter of hours or days at room temperature. Data are presented for linearity, and analytical applications are suggested.

THE quantitative determination of small amounts of dissolved water in an organic liquid by chemical means, whether in industry or in the laboratory, is usually very time-consuming and instantaneous results cannot be obtained in continuous-flow systems. The need for a speedier method has been reflected of late in the increasing resort to dielectric measurements for inferring moisture content in various liquid-liquid solutions. An important industrial product, ethyl acetate, was suggested for study of moisture content by this means (*1*). No previous data have been published on dielectric value *vs.* dissolved moisture for this compound. The call for such data is thus easily surmised.

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EXPERIMENTAL

The heterodyne-beat method, employing a C-R oscilloscope to show an unequivocal Lissajous figure at the end point, was used for determining the test-cell capacitance—i.e., dielectric values—and Karl Fischer reagent with dead-stop end point was utilized for corresponding moisture determinations. The arrangement of the apparatus is shown in Figure 1.

With so sensitive a null-point arrangement, much care was taken to assure stability in the oscillators. The Clapp circuit (*3*) was used in the variable oscillator, and the crystal oscillator was based on the Colpitts circuit (*7*) modified to be additionally stable.

Test Cells. In neither of two cells which were made was there any departure in principle from the conventional form of having two capacitor plates, as seen in Figure 1. One cell, water-jacketed for constant temperature and allowing total immersion of the plates (*6*), was made to serve as a measure of the purity of the starting-point, or standard, benzene and to check for dielectric value against that published by the National Bureau of Standards (*5*). No further use for this cell was called for.

A second cell, the test cell, was also water-jacketed for constant temperature.

PROCEDURE

When filled with standard benzene (ϵ = 2.284) (*4*), the test cell constant was determined with the aid of a calibrated variable condenser. Thereafter, all dielectric data were obtained by replacing the benzene with the ethyl acetate test sample, whose water content had been ascertained by Karl Fischer reagent. Each point of the final graph of dielectric value *vs.* moisture content (Figure 2) was the average value of several determinations of the dielectric value of the sample concerned. Starting with anhydrous ethyl acetate, moisture content was increased by stepwise additions of wet ethyl acetate, with prolonged shaking. All Fischer titrations were performed under small positive pressure of dry air, to ensure absence of atmospheric moisture.

PURIFICATION OF REAGENTS

Distillations were performed in all-glass apparatus, under slight positive pressure of air dried over phosphorus pentoxide.

Ethyl acetate, obtained from British Drug Houses (Canada), Ltd., was dried over phosphorus pentoxide, distilled, and stored in tightly stoppered flasks. No water was detectable by Karl Fischer reagent, good for less than 0.01% moisture. Measured specific gravity was 0.9037 at 21.6° C.; published value, 0.901 at 20° C. (*2*).

1,4-Dioxane (for Karl Fischer titrations), also from British Drug Houses, was refluxed for several hours over sodium, distilled, and stored over sodium. No moisture was then detectable. Measured specific gravity was 1.03; published value, 1.035 (*2*).

Benzene was used as the reference standard. It was shaken extensively with concentrated sulfuric acid, then with distilled water, and finally with sodium carbonate solution. After drying over calcium chloride, it was distilled and stored over sodium. No moisture was then detectable in the benzene. Measured specific gravity and refractive index at 24° C. were 0.872 and 1.502, respectively; published values, 0.879 and 1.50112 at 20° C. (*2*).

RESULTS AND DISCUSSION

The linear relationship shown by Figure 2 results from determinations made not later than an hour after each addition of water, as it had been determined that slow hydrolysis would superimpose an undesired side effect if the solution were allowed to stand for extended periods.

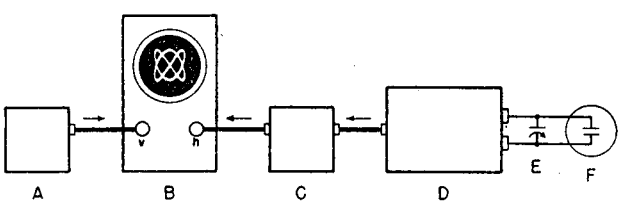

Figure 1. Schematic electronic circuit for determination of dielectric values (constants)

A. Crystal oscillator of fixed frequency, 400 kc. per second
B. Cathode-ray oscilloscope detector
C. Buffer amplifier, for better electrical isolation between *A* and *D*
D. Variable-frequency oscillator (600 kc. per second at null point)
E. Tuning condenser of *D* (actually housed within *D*, but shown schematically outside)
F. Capacitor test cell, temperature controlled

So great is the difference between the dielectric constants of water (80.4) and ethyl acetate (6.02) that dielectric measurement vies favorably with Karl Fischer titration in the accurate determination of moisture in ethyl acetate, up to at least 1.7% water, and probably also up to the limit of solubility—i.e., about 3.3% water (*4*).

Any wandering of points in Figure 2 is considered to be a reflection of the difficulty of obtaining reliable end points in the Karl Fischer determinations; it is not considered attributable to the electronic method made use of in measuring dielectric values in this work.

CONCLUSIONS

It is suggested by this study that ethyl acetate may be analyzed for moisture content by dielectric means. This analysis is comparatively rapid; but, with a continuously indicating dielectric meter or recorder, instantaneous analyses could be had for either batch or continuous-flow systems. It is not to be denied that the accuracy of this method would be influenced by fluctuations in the temperature of the liquid, in common with temperature-volume relationships of all liquids.

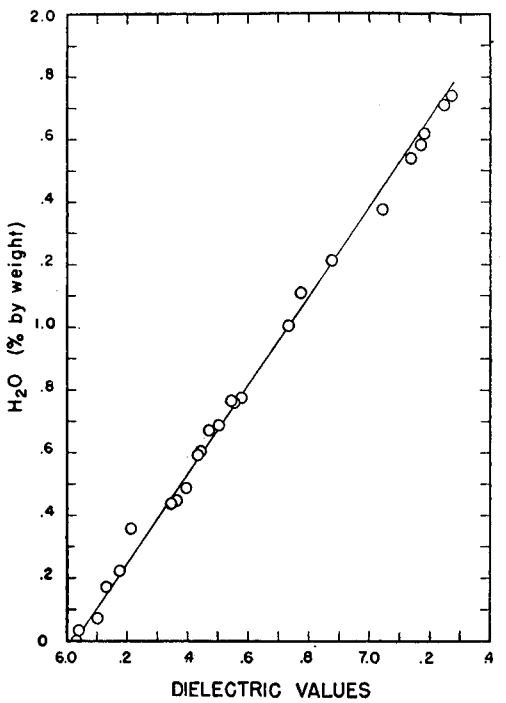

Figure 2. Linear relationship between dielectric values and moisture dissolved in ethyl acetate, at 20° C.

Each point represents determinations made upon a freshly prepared sample

ACKNOWLEDGMENT

The authors acknowledge their indebtedness to Shawinigan Chemicals, Ltd., for the suggestion that motivated this study.

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RECEIVED for review February 15, 1956. Accepted April 12, 1956.

Photometric Determination of Nickel in Petroleum Cracking Catalyst

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A rapid method was required for detecting nickel contamination in petroleum cracking catalyst. None of the proposed procedures using dimethylglyoxime was satisfactory for this particular purpose, mainly because the proper conditions of pH and the control of interferences were not described. The proposed method gives excellent reproducibility, is simple, and compares favorably with the lengthy gravimetric procedure. The color is stable and no precipitation of nickel occurs in the range up to 300 γ of nickel per gram of sample.

THE determination of nickel in petroleum cracking catalyst is significant because the nickel is a catalyst poison and also a very likely contaminant in oil feed stocks. Dimethylglyoxime has long been used as the specific reagent for gravimetric determinations, and procedures have been proposed for its use in the photometric determination of nickel. In this laboratory none of these procedures was successfully applied to petroleum cracking catalysts. Therefore, a procedure was developed for the photometric determination of nickel by dimethylglyoxime, with improvements to give more stable color in a wider range. Control of interfering substances is achieved by the use of tartaric acid and phosphoric acid (*3*) to complex iron and alumina (*2*), and by measurement at a wave length of 530 mμ. A stable color is obtained and losses due to decomposition are controlled by using bromine (*1*) as an oxidation agent and by adding the dimethylglyoxime under the proper conditions of pH and temperature.

The procedure is applicable in the range from 10 to 300 γ of nickel and the colors are stable for more than 24 hours (*2*). The absorbance of the colored complex nickel compound (*5*) may be read in a Beckman DU spectrophotometer at 530 mμ or by use of No. 525 filter in a Fisher Electrophotometer or nephelometer. Interference from cobalt and iron is of little consequence at this wave length (*2*).

PROCEDURE

Reagents. The following reagents, all analytical reagent grade chemicals, are required: hydrofluoric acid, sulfuric acid, nitric acid, phosphoric acid, tartaric acid, ammonium hydroxide, sodium hydroxide, a saturated water solution of bromine, and a 1% alcoholic solution of dimethylglyoxime.

Standard nickel solution. Dissolve 0.1002 gram of pure metallic nickel in nitric acid with boiling. Add 2 ml. of sulfuric acid, evaporate until fumes of sulfur trioxide are evolved, and dilute to volume in a 1-liter volumetric flask. Transfer 50 ml. of this solution to a 500-ml. volumetric flask and dilute to volume. One milliliter of the final solution should contain 10 γ of nickel.

Preparation of Standard Curve. Prepare a series of solutions in 100-ml. beakers to contain 0, 10, 20, 30, 50, 100, 200, and 300 γ of nickel. To each add 5 ml. of a 20% solution of tartaric acid, 2 ml. of 1 to 4 phosphoric acid, and 2 ml. of 1 to 1 sulfuric acid. Dilute to 50 ml. with water and add 5 ml. of a saturated water solution of bromine. Mix and allow to stand for at least 1 minute. Add 1 to 1 ammonium hydroxide dropwise, meanwhile stirring until excess bromine is destroyed as shown by clearing of the color of the solution. Add a 2-ml. excess of 1 to 1 ammonium hydroxide and cool in a water bath below 20° C. Raise the pH of each solution to 11.5 ± 0.5 by the addition of 6*N* sodium hydroxide, and immediately add 2 ml. of 1% dimethylglyoxime solution. Transfer the solutions to 100-ml. volumetric flasks, dilute to volume, mix, and, after 15 minutes, read the absorbance at 530 mμ. Plot readings on linear graph paper against micrograms of nickel.

Preparation of Samples. Transfer from 2 to 5 grams of sample to a 100-ml. platinum dish and wet with water. Add hydrofluoric acid in small increments to decompose the silica, and finally evaporate to dryness. Add 5 ml. of sulfuric acid and fume strongly for 15 minutes to expel excess fluoride. Cool, transfer the sample to a 250-ml. volumetric flask, and boil for 5 minutes. Cool, dilute to volume with water, and mix. Filter through a dry Whatman No. 40 paper and transfer an aliquot containing up to 300 γ of nickel to a 100-ml. beaker. Proceed exactly as described for preparation of the standard curve, omitting only the addition of the sulfuric acid.

EXPERIMENTAL

In the initial work on the procedure, erratic results were obtained. Frequently no color development took place and at times the colors which developed ranged from brown to red with a stability that ranged from seconds to days. Precipitation often took place even in very low concentrations of nickel.

Tests were made at many pH ranges and with various quantities of bromine, ammonia, sodium hydroxide, and dimethylglyoxime. It was found that no difficulty was encountered with the proposed procedure, which gives a stable color for days and no precipitation within the range specified.

Table I. Comparison of Gravimetric and Photometric Procedures for Nickel

Nickel Found, %		
Gravimetric	Photometric	Diff., %
0.096	0.097	+0.001
0.093	0.096	+0.003
0.092	0.092	...
0.086	0.081	−0.005
0.082	0.086	+0.004
0.079	0.085	+0.006
0.073	0.072	−0.001
0.061	0.054	−0.007
0.017	0.015	−0.002
0.015	0.011	−0.004
0.013	0.011	−0.002
0.011	0.010	−0.001
0.009	0.007	−0.002
0.008	0.009	+0.001
0.005	0.005	...

RESULTS AND DISCUSSION

Fifteen different samples of regenerated petroleum cracking catalysts were tested by both the gravimetric and photometric procedures. The results are shown in Table I.

The chief advantages of this method are its simplicity and rapidity. No separations are necessary and, thus, few errors are introduced. With proper planning and some experience, a test may be run in 2 hours; the procedure is also adaptable to the simultaneous determination of several samples. While little exploratory work has been done, the authors believe the method can be used on a wide variety of materials.

ACKNOWLEDGMENT

The authors wish to acknowledge the help and information found in E. B. Sandell's book (*4*).

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RECEIVED for review November 1, 1955. Accepted April 16, 1956. Presented at meeting of Maryland Section, ACS, John Hopkins University, Baltimore, Md., November 1955.

129. Uranium Monochlorotrifluoride, $UClF_3$

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Crystals of uranium monochlorotrifluoride were prepared by A. W. Savage of this laboratory by four different methods (*2*).

1. By the reaction of uranium trifluoride with chlorine at 350° C. This method was used by Warf and others (*1*), who described the product as isometric on the basis of powder x-ray diffraction patterns. Although the symmetry of this compound is orthorhombic, it is so nearly isometric metrically that powder x-ray diffraction patterns can be interpreted on the basis of a simple isometric unit cell.
2. By the reaction of uranyl fluoride (UO_2F_2) with carbon tetrachloride at 450° C. This method was used by Gates and others (*1*), who assumed the reaction products to be uranium dichlorodifluoride (UCl_2F_2), carbonyl chloride ($COCl_2$), and chlorine. Considerations of crystal chemistry preclude the possibility that the principal solid product of this reaction is uranium dichlorodifluoride.
3. By heating uranium tetrafluoride with uranium tetrachloride in a neutral atmosphere.
4. By heating uranium tetrafluoride with carbon tetrachloride in a neutral atmosphere.

None of these methods yielded a product consisting of a single crystalline phase. An analysis of a product of the reaction of uranyl fluoride with carbon tetrachloride, estimated to contain 95% uranium monochlorotrifluoride, gave (by weight) 70.6% uranium, 10.7% chlorine, and 20.3% fluorine. This corresponds to the atomic ratio U:Cl:F = 1:1.01:3.60.

Absorption Spectrum

(Band maxima in millimicrons and relative intensities as viewed with Zeiss prism microspectrometric eyepiece)

Parallel to *X*	Parallel to *Y* and *Z*
660 very strong	675 strong
655 weak	663 weak
645 weak	650 very strong
640 strong	638 strong
633 weak	630 medium strong
620 strong	590-620 medium
608 weak	
583 medium strong	
	522-538 weak
527 medium strong	531 medium strong
	522 medium
495 weak	495 medium, wide
470 weak	470 medium, wide

Partial Powder X-Ray Diffraction Pattern

$h^2 + k^2 + l^2$	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
3	5.009	4.95	5
4	4.331–4.345	4.30	100
5	3.885	3.85	40
6	3.541	3.52	65
8	3.065–3.069	3.05	70
9	2.892	2.88	45
11	2.613–2.619	2.60	20
12	2.504	...	..
13	2.407	...	..
14	2.317–2.321	2.31	45
16	2.166–2.173	2.16	40
17	2.104–2.107	2.09	35
18	2.044	2.04	70
19	1.989–1.991	1.984	95
20	1.938–1.942	1.933	45
21	1.892–1.895	1.885	80
22	1.850	...	..
24	1.770–1.772	1.767	10
25	1.737	...	..
26	1.700–1.702	1.697	55
27	1.668–1.672	1.664	50
29	1.610–1.612	1.606	5
30	1.582–1.586	1.579	25
32	1.532–1.535	...	..
33	1.510	1.506	35
34	1.488	...	..
35	1.466–1.468	1.463	45
36	1.444–1.448	1.444	30
37	1.429	1.430	<5
38	1.407–1.408	1.403	5
40	1.370–1.374	1.368	30
41	1.355–1.357	1.353	30
42	1.337–1.340	1.336	30
43	1.323–1.324	1.320	65
44	1.306–1.309	1.305	20

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(CuK\alpha) = 1.5418$ A.
[b] Relative peak intensities above background from densitometer measurements.

Crystal Morphology

System and Class. Orthorhombic, dipyramidal.

Habit. Tabular {001} or dipyramidal {111} with {001} and {100}.

Polar Angles. (111) Λ (100) = 53½° (measured), 54° 44′ (calculated from cell dimensions); (111) Λ (001) = 52½° (measured), 54° 44′ (calculated).

X-Ray Diffraction Data

Diffraction Symbol. *mmmAba-*, embracing space groups *Abam* (D_{2h}^{18}) and *Aba2* (C_{2v}^{17}).

Cell Dimensions (from Calibrated Weissenberg and Precession Photographs). $a_0 = 8.673 \pm 0.002$ A., $b_0 = 8.690 \pm 0.01$ A., $c_0 = 8.663 \pm 0.005$ A.; $a:b:c = 0.998:1:0.997$. Cell volume 652.9 A³.

Formula Weights per Cell. 8.

Formula Weight. 330.53.

Density. 6.72 grams per cc. (calculated from cell dimensions; weight of unit atomic weight 1.6602×10^{-24} gram).

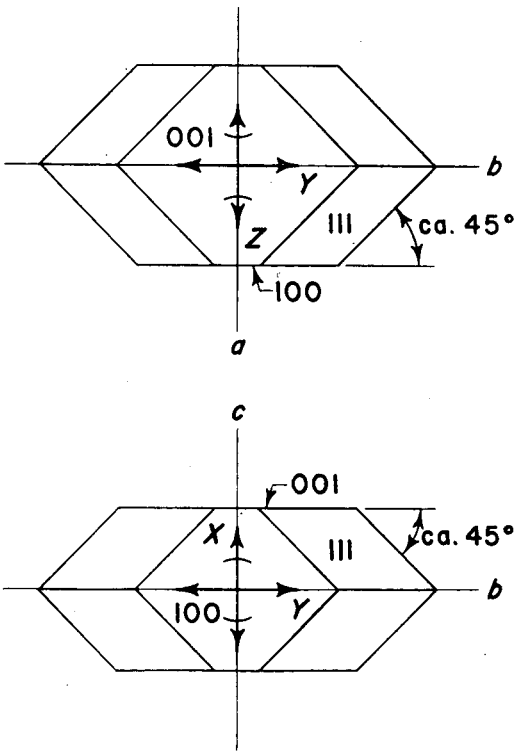

Figure 1. Orthographic projection of crystal of uranium monochlorotrifluoride on (100) and (001)

Optical Properties

Refractive Indices (5893 A.). $n_X = 1.727$, $n_Y = 1.745$, $n_Z = 1.757$, all ± 0.005; geometric mean 1.743. Molecular refraction 19.86 cc.

Optic Orientation. $X = c, Y = b, Z = a$.

Optic Axial Angle (5893 A.). $2V_X = 80 \pm 5°$; $r < v$, weak.

Color. Green with absorption $Z > Y > X$.

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Work done under the auspices of the Atomic Energy Commission.

130. Uranyl Carbonate, UO_2CO_3

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Uranyl carbonate was prepared by B. J. Thamer of this laboratory by reaction of uranium trioxide with water and carbon dioxide at 300° C. for 1 week. Rutherfordine, a naturally occurring uranyl carbonate, has been described (*2*).

Crystal Morphology

System and Class. Orthorhombic, dipyramidal.

Axial Elements. $a:b:c = 0.526:1:0.466$ (derived from unit cell dimensions).

Habit. Rectangular {010} plates, usually elongated [001], bounded by {100}, {001}. Cleavage {010}.

X-Ray Diffraction Data

The structure of rutherfordine has been determined by Christ, Clark, and Evans (*1*). The space group is $Pmmn$ (D_{2h}^{13}).

Cell Dimensions. $a_0 = 4.85$ A.; $b_0 = 9.22$ A.; $c_0 = 4.30$ A. Cell volume 192.3 A.^{3}. Christ (*1*) reported $a_0 = 4.845 \pm 0.010$ A.; $b_0 = 9.205 \pm 0.008$ A.; $c_0 = 4.296 \pm 0.006$ A.

Formula Weights per Cell. 2.

Formula Weight. 330.08.

Density. 5.70 grams per cc. (x-ray).

Partial Powder X-Ray Diffraction Pattern of Uranyl Carbonate

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
020	4.61	4.56	100
110	4.29	4.25	35
011	3.90	3.86	30
101	3.22	3.19	35
121	2.638	2.62	25
130	2.596	2.58	10
031	2.500	2.48	10
200	2.425	2.41	10
040	2.305	2.29	30
002	2.150	2.14	20
220	2.147		
211	2.059	2.05	30
022	1.949	1.940	10
112	1.922	1.913	20
141	1.874	1.866	15
231	1.741	1.735	15
150	1.724	1.715	15
051	1.695	1.688	10
240	1.671	1.664	10
132	1.656	1.648	10
202	1.609	1.601	5
310	1.592	1.586	5
042	1.572	1.565	5
060	1.537	1.529	10
222	1.519	1.509	10
301	1.513		
321	1.438	1.431	10
330	1.431		
013	1.416	1.409	5
251	1.389	1.383	20
161	1.387		

[a] Philips 114.6-mm.-diameter powder camera; Straumanis mounting; $\lambda(CuK\alpha) = 1.5418$ A.

[b] Relative peak intensities above background from densitometer measurements.

Optical Properties

Refractive Indices (5893 A.). $n_X = 1.70 \pm 0.01$; $n_Y = 1.716 \pm 0.003$; $n_Z = 1.795 \pm 0.005$; geometric mean 1.737. Molecular refraction 23.3 cc. For rutherfordine, Larsen found $n_X = 1.72$; $n_Z = 1.80$ (*2*).

Optic Orientation. $X = b$; $Y = c$; $Z = a$.

Optic Axial Angle. $2V_Z = 46°$.

Color. Yellow. No observable fluorescence was excited by a mercury vapor lamp.

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Work done under the auspices of the Atomic Energy Commission.

131. Uranium(IV) Pyrophosphate, UP_2O_7, Orthorhombic Form

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Two polymorphic modifications of uranium(IV) pyrophosphate are described in Gmelin (*1*): (1) isometric, octahedral or cubic habit, white with slight rose or brown coloring, optically isotropic; and (2) orthorhombic, tabular habit, green, transparent, optically anisotropic. The structure of the isometric form has been described by Peyronel (*3*), but crystallographic data for the orthorhombic form have been limited to those given above.

Excellent crystals of the orthorhombic form were grown from an 87% phosphoric acid solution at 400° C. by B. J. Thamer of this laboratory. Analysis of a somewhat impure sample gave (by weight) uranium 54.5%, phosphorus 14.9%, corresponding to the atomic ratio P/U = 2.10 (calculated for UP_2O_7, by weight, uranium 57.78%, phosphorus 15.04%). The crystals are stable in air and are apparently unaffected by hot or cold, concentrated or dilute nitric, hydrochloric, and sulfuric acids and aqua regia.

Crystal Morphology

System and Class. Orthorhombic, probably dipyramidal. No piezoelectric effect was detected with a Giebe-Scheibe–type apparatus.

Axial Elements. $a:b:c = 0.88:1:?$ (measured), $0.900:1:0.550$ (calculated from cell dimensions).

Habit. Tabular parallel to {010}, with {110} and {001} less prominent.

Twinning. Not observed.

Cleavage. {010} prominent; also {001} and {110}.

Polar Angles. (010) ∧ (110) = 48° 38′ (measured), 48° 01′ (calculated from cell dimensions).

X-Ray Diffraction Data

Diffraction Symbol. *mmmPna-*, embracing space groups $Pnam$ (D_{2h}^{16}) and $Pna2_1$ (C_{2v}^{9}).

Cell Dimensions (from Calibrated Weissenberg Photographs). $a_0 = 11.526$, $b_0 = 12.810$, $c_0 = 7.045$ A., all ± 0.005 A. Cell volume 1040.2 A.^{3}.

Formula Weights per Cell. 8.

Formula Weight. 412.03.

Density. 5.26_1 grams per cc. (calculated; weight of unit atomic weight 1.6602×10^{-24} gram); 5.22_8 (measured pycnometrically).

Optical Properties

Refractive Indices (5893 A.). $n_X = 1.734 \pm 0.003$, $n_Y = 1.79_1$ (calculated from optic axial angle), $n_Z = 1.796 \pm 0.003$; geometric mean 1.773. Mean refractive index calculated from ap-

proximate specific refractive energies [Larsen and Berman (2)] and calculated density, 1.79. Molecular refraction 32.6 cc.

Optic Orientation. $X = c$, $Y = b$, $Z = a$.

Optical Axial Angle (5893 A.). $2V_X = 33^1/_2 \pm {}^1/_2°$; $r < v$, moderate.

Color. Emerald green with moderate absorption $Z \backsimeq Y > X$.

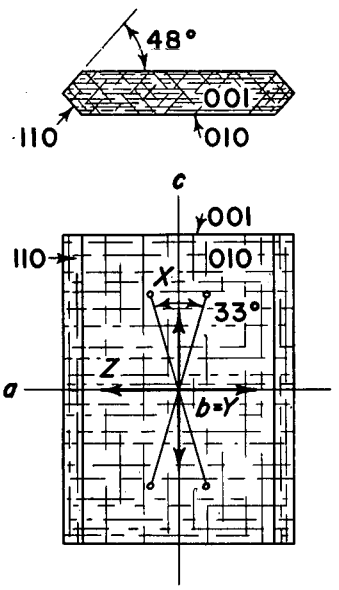

Figure 1. Orthographic projections of crystal of uranium (IV) pyrophosphate, orthorhombic form, on (010) and (001)

Partial Powder X-Ray Diffraction Pattern of Uranium(IV) Pyrophosphate

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
110	8.569	...	..
020	6.405	6.35	55
011	6.116	...	..
200	5.763	5.74	5
120	5.598	5.56	5
111	5.466	5.42	<5
210	5.256	5.24	50
201	4.461	...	..
121	4.383	4.36	60
220	4.284	4.26	20
211	4.213	4.20	25
130	4.004	4.00	5
310	3.680	3.67	50
221	3.660	...	..
031	3.651	...	..
002	3.523	3.52	100
131	3.481	3.47	15
230	3.431	3.42	10
320	3.295	...	..
311	3.272	...	..
112	3.258	3.25	10
040	3.203	3 19	10
122	3.087		
140	3.086	3.08	35
231	3.084		
202	3.006	2.988	5
122	2.981	...	..
321	2.980	...	..
212	2.926	2.921	20
400	2.881	...	..
330	2.856	2.849	35
141	2.826	2.820	45
410	2.811	...	..
240	2.799	...	..
222	2.721	2.714	<5
401	2.667	2.653	10
331	2.647	2.640	25
132	2.645		
420	2.628	...	..
411	2.611	2.607	35
241	2.601		

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; $\lambda(CuK\alpha) = 1.5418$ A.

[b] Relative peak intensities above background from densitometer measurements.

Absorption Spectrum (Band Maxima in Millimicrons and Relative Intensities as viewed with Zeiss Prism Microspectrometric Eyepiece). Parallel to X: 665 (strong), 645 (strong), 626 (strong), 612 (strong). Parallel to $Z \backsimeq Y$: 648 (very strong), 620 (strong), 603 (medium, very wide), 585 (medium, wide), 570 (weak, very wide), 530 (weak, very wide), 490 (medium, very wide).

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WORK done under the auspices of the Atomic Energy Commission.

MEETING REPORT

Society for Analytical Chemistry

A MEETING was held May 2 in London, where a paper on "Composition of Some Deposits and Muds in Estuaries, Rivers, and Lakes" was presented by J. H. Hamence, Bernard Dyer & Partners, Ltd., 20 Eastcheap, London E. C. 3.

Deposits and muds in estuaries, rivers, and lakes were examined to determine their origin and to detect material causing pollution. The following aspects were considered.

Methods of sampling and preparation of material for analysis, whereby gross impurities are removed.

Value of a preliminary microscopical examination for detection of algae, and characteristic organic tissues.

Proportion of water, organic matter, and mineral matter in deposits of widely different origin. Without other criteria, these figures are of little value in establishing pollution.

Clearing mud for identifying specific minerals causing pollution.

Application of mechanical analysis, used in soil analysis to determine particle size distribution.

Chemical composition of mineral matter in deposits, and comparison with the general chemical composition of mineral matter in soils.

Proportions of nitrogen and phosphorus in muds, in connection with detection of pollution due to sewage.

Except in special cases, where substantial growths of algae are present, the proportion of nitrogen in the organic matter in deposits falls within well defined narrow limits, which are the same whether sewage is present or not. Clearly some mechanism operates in muds similar to those in arable soils to keep the carbon-nitrogen ratio more or less constant.

While the proportion of nitrogen present is no guide as to the presence of sewage, muds from sewage-polluted sources show high phosphorus contents.

The Wakeley-Black method used in soil analysis for determining the carbon-nitrogen ratio needs further investigation before it can be taken as providing a true indication of the carbon content of muds. Many muds have been examined for the presence of trace metals, including lead, copper, and zinc. Copper is present in substantial quantities in mud from sewage-polluted rivers and estuaries; high copper in mud may usually be taken as indicative of sewage pollution.

Lead has been found in the majority of muds in substantial amounts. The theory that this is mainly the result of atmospheric pollution appears to be supported by the examination of the contents of atmospheric deposit bowls.

Zinc is also a common constituent of muds and its quantity increases with sewage pollution. Part of the zinc is derived from atmospheric pollution. The manner in which these metals, etc., are adsorbed on the suspended matter, carried down in the river, and deposited was discussed.

The nature of the ether-soluble matter in mud has been investigated in connection with the detection of pollution due to mineral oil. Detecting mineral oil involved chromatographic separation and led to a large scale chromatographic separation of the ether-soluble matter from sewage sludge. This separation revealed the presence of a number of different substances which gave characteristic fluorescent colors under ultraviolet light. A similar examination of the ether-soluble matter from soils showed pigments common to both which exhibit similar fluorescence under ultraviolet.

A chromatographic technique has been derived which will distinguish between natural deposits or muds and deposits containing sewage solids.

Ebullition Device

Takeo Iida, Pharmaceutical Institute, University of Toyama, Toyama, Japan

THREE new types of ebullition tube to prevent bumping have been devised by utilizing porous glass as an ebullition nucleus: a porous glass rod for ordinary pressure distillation, a porous glass capillary for reduced pressure, and a distillation flask with a porous glass protruding tip for both pressures. The tubes have performed satisfactorily for the past few years in this laboratory.

POROUS GLASS ROD

As shown in Figure 1, *a*, a piece of glass tube is fused and kneaded into an S-shape several times, so as to allow the occlusion of air bubbles. This is twisted into a lump, *b*, and rapidly stretched into a porous glass rod, *c*. *c* contains many blind capillaries formed by the stretching operation, as is shown in the enlarged cross section, *f*, and it glistens like strands of silk and is brittle. This porous rod is fused on a microflame, stretched at 20- to 25-mm. intervals, *d*, and then is cut in the middle of the stretched portion, affording two porous glass rods, *e*. This porous glass rod is employed as an ebullition nucleus for ordinary pressure distillation, as shown in *g* and *h*. Solvents (ether, ethyl alcohol, benzene, chloroform, etc.) boil smoothly without bumping on a water bath, *g*, and oils of high boiling point can be distilled without difficulty on the flame, *h*.

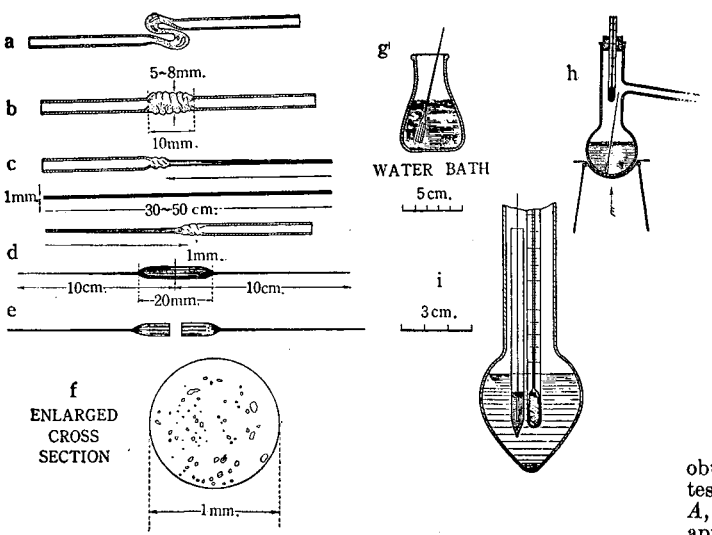

Figure 1

E. Hayashi and H. Yamanaka, Shizuoka Pharmaceutical College (private communication), used this porous glass rod successfully as an ebullition nucleus in semimicrodeterminations of boiling point [A. Siwoloboff, *Ber.* 19, 795 (1886)], as shown in *i*. The boiling point of a liquid is determined distinctly when continuous bubbling from a porous glass rod is observed. The following shows the boiling points (° C., uncorrected) of several liquids determined using the porous glass rod.

Acetone	56.5
Chloroform	60.0– 60.5
Benzene	80.5
Toluene	110.3–110.5
Chlorobenzene	131.5–132.0
Aniline	183 –184
Dimethylaniline	192 –193
Guaiacol	203 –203.5
Quinoline	234 –234.5

Repeated experience has shown that when boiling is stopped, and the solution is allowed to cool and is then reheated, this rod continues to provide fine boiling nuclei, unlike other types of ebullition tubes employed hitherto.

POROUS GLASS CAPILLARY

As shown in Figure 2, a glass tube, *A*, containing two or three capillaries of the type employed for melting point determination, is fused together and stretched.

A porous glass tube, *b*, is obtained having several pores penetrating into the interior. At the point of desired length the stretched tube is again melted over a microflame and stretched rapidly; a porous glass capillary, *c*, possessing very small pores is

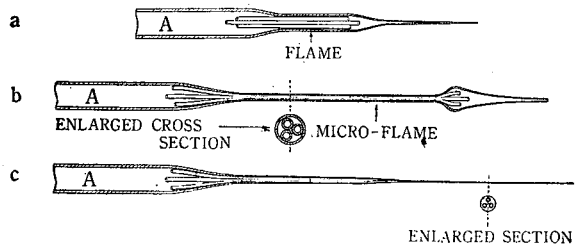

Figure 2

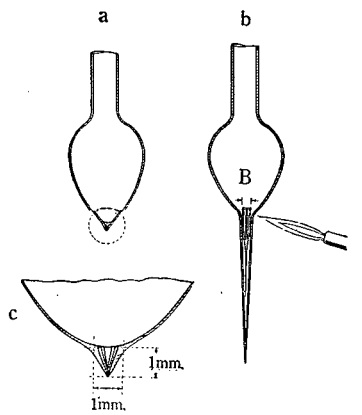

Figure 3

obtained, as shown by the enlarged cross section. In order to test its suitability, a rubber bulb is attached at one end of tube *A*, the capillary placed under the surface of water, and pressure applied to the bulb; if fine bubbles form from the end of the capillary, it is of the desired size for use in vacuum distillation.

This porous glass capillary is substituted for the single-bore capillary with advantage. The single-bore capillary used to date in low-pressure distillations is made with a very fine bore in order to obtain minute bubbles, and this requires a highly skilled technique and is often inconvenient for amateurs to manipulate. The present tube is very easy to prepare and convenient in handling, because it is as strong as a fine rod and is well suited as an ebullition tube for a large distillation flask. The porous glass tube can be repeatedly used by melting and pulling into the porous glass capillary.

DISTILLATION FLASK WITH POROUS GLASS PROTRUDING TIP

The porous glass capillary or rod is cut into pieces 2 to 3 cm. in length, soaked in a concentrated aluminum sulfate solution, dried, and then heated in the flame until the pores of the capillary are covered with an alumina layer. The alumina layer prevents diminution of the pores when it is being sealed into the bottom of the flask and when the resinous matter accumulated in the pores

during the distillation is burned to refresh the tip. Capillary *B* (Figure 3) is flush with the bottom of flask *a;* a porous glass protruding tip, shown by the enlarged figure, *c*, is obtained.

It is desirable to carry out distillation by heating over a free flame rather than in a bath, as heating in a bath does not always give satisfactory results. Therefore distillation flask *b* should be made of hard glass such as borosilicate glass. When the flask is of glass with a large coefficient of expansion, the protruding tip may break off or the flask develop a crack during distillation.

This distillation flask provides an excellent compact ebullition mechanism. The bottom of the tube will always be heated above the boiling point of the liquid in the flask, so that vapor bubbles evolved form the ebullition nucleus. This is also true in the case of low pressure. Therefore reduced pressure distillation with this flask can be carried out without help of a capillary. For example, an ether solution may be distilled off at atmospheric pressure and the ether residue distilled immediately at a reduced pressure, without changing flasks or introducing a capillary tube. This protruding glass tube utilizes the vapor itself as the ebullition nucleus and does not require air bubbles for its continued operation. Therefore it is also possible to distill in a high vacuum.

Method of Degassing Liquids

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In experiments on the radiolysis of alcohols and ethers using very low energy input, and therefore with a product yield of only a few micromoles, traces of air in the liquids markedly influenced the results. The quantitative recovery of very small amounts of gaseous products, especially the higher boiling hydrocarbons such as propane and the butanes, was difficult. In order to simplify and speed both the original deaerating and product recovery, a method has been devised whereby the liquid can be refluxed under vacuum while pumping on it either with a regular vacuum pump for deaerating or with a Toepler pump for collecting dissolved gases.

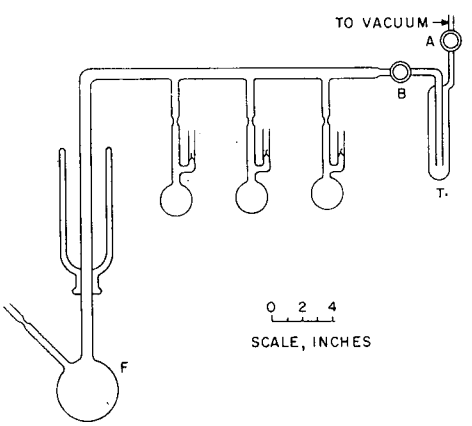

Figure 1

In essence, the apparatus is a low-temperature reflux condenser, in which the liquid can be refluxed at a temperature at which its vapor pressure is negligible (<1 mm.). The method for deaerating is limited to materials that have low vapor pressures at temperatures above their melting points, thereby excluding such compounds as water, benzene, and glacial acetic acid. In principle, dissolved gases can be determined in these high-melting liquids by forming a low-melting eutectic with a previously degassed liquid of lower boiling point and refluxing this mixture under vacuum. This has been illustrated with benzene.

EXPERIMENTAL

Degassing Liquids. The apparatus used for refluxing liquids under vacuum is shown in Figure 1.

It was set up with the receiving flasks sealed in place on the line. The liquid to be degassed was introduced into flask *F*, sealed off with the usual precautions, and refluxed under vacuum using a dry ice–trichloroethylene slurry in the condenser and a liquid nitrogen trap at *T*. Precooling flask *F* is an aid in avoiding bumping during the initial phases of the evacuation. Pumping was continued until the pressure in the connecting vacuum system was down to less than about 1 micron (as measured by a vacuum thermocouple gage). The pressure in the vacuum system beyond the nitrogen trap showed no change when stopcock *A* was turned off for 10 or 15 minutes and then opened. For 250 ml. of liquid, starting at atmospheric pressure, this process required from 1 to 2 hours. The initial phases of the degassing can be further speeded by stirring the liquid in flask *F* with a magnetic stirrer.

Stopcock *B* was then turned off, the dry ice removed from the condenser with a long-handled spoon and a precooled syringe, and the liquid distilled into the final receivers with dry ice cooling. *F* is cooled with dry ice and *B* is opened while each receiver is being sealed off.

The system is not limited to dry ice cooling; any cooling agent that gives a temperature above the melting point of the solvent, yet low enough to reduce the vapor pressure to a reasonable value, may be used. A fairly high vapor pressure can be tolerated if the size of the liquid nitrogen trap is increased and some loss of material can be tolerated. A carbon disulfide slush bath (melting point −110° C.) has been used successfully in degassing diethyl ether.

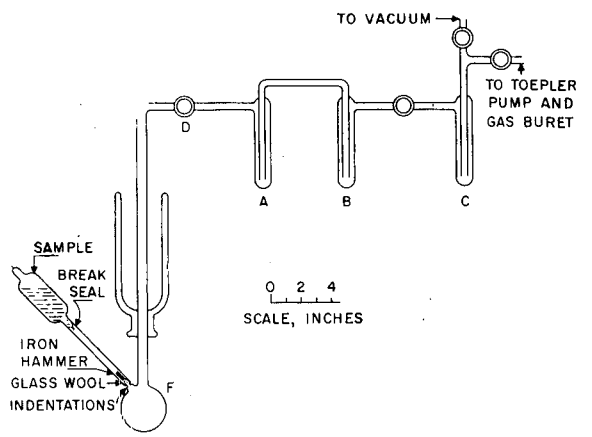

Figure 2

Recovery of Gases from Liquids. The system for the quantitative recovery of dissolved gases from liquids is essentially the same, but if gases as high boiling as butanes are to be recovered, an additional step is necessary. The apparatus is shown in Figure 2.

The gas sample was sealed to the side arm of flask *F* (irradiated samples are contained in flasks with break seals and contain high-boiling "polymers" which are later recovered from residual material in flask *F*, hence the vertical placement of the sample flask for complete drainage into flask *F*) and the system was thoroughly evacuated. The condenser was then filled with dry ice and trichloroethylene slush, flask *F* was cooled with dry ice, stopcock *D* was closed, and the sample was introduced to *F*. The glass-wool plug served as a shock absorber for the iron hammer on its return.

Liquid nitrogen traps were placed around A and B, the dry ice bath was removed from F, and the noncondensable gases were collected by the Toepler pump, while all condensable gases, together with a small amount of solvent, were collected in trap A. When no further gas can be collected through the liquid nitrogen traps, any higher boiling gas can be fractionated from the small amount of material collected in A. The collection of material from F into A should be continued until the residue in F will reflux indefinitely with stopcock D closed. Further removals may be done intermittently while the next step is in progress.

The distillate in A may be cut into as many fractions as desired by distillation from A to B, with liquid nitrogen pumping at C (*2*), using any desired temperature bath on B to regulate the composition of the cut. Using a carbon dioxide–trichloroethylene bath on B, three trap-to-trap distillations have been found adequate to isolate butanes quantitatively from alcohols and ethers. Between distillations if flask F stops refluxing, the volatile material may be transferred to A while the material in B is being returned to A. A trace of solvent is always distilled to C in the transfer from A to B, if the vapor pressure of the solvent is at all appreciable at the temperature of B.

As a check on this procedure, 0.126 millimole of a mixture of isobutane, isobutene, propane, and propene (Phillips Petroleum Co., research grade hydrocarbons) was added to 50 ml. of degassed isopropyl ether [freezing point −85.7°; literature (*1*), −85.89°] by liquid nitrogen transfer from a gas buret. It was degassed by the procedure outlined above (Figure 2) and the gases from the Toepler pump were collected over mercury in a gas buret. The added gas and the final recovered gas were analyzed using a Consolidated Engineering Corp. Model 21-103 mass spectrometer. The recovery is shown in Table I.

Table I. Recovery of Gases from Diisopropyl Ether

Component	Millimole Added	Millimole Found[a]	Δ Millimole
Propene	0.0482	0.0485	+0.0003
Propane	0.0320	0.0323	+0.0003
Isobutene	0.0309	0.0296	−0.0013
Isobutane	0.0149	0.0156	+0.0007
Total	0.126	0.126	

[a] Recovered gas also contained 0.0114 millimole of diisopropyl ether (8.3% of total gas collected).

For comparison with one usual method of degassing (pumping at low temperature followed by partial evaporation), 100 ml. of diisopropyl ether was saturated with methane at room temperature, then degassed by cooling with dry ice and pumping on it for 30 minutes. The ether was further divided into two parts by vacuum distillation of half of it into a second dry ice–cooled trap consisting of a U-trap in the vacuum line with a volume at the bottom of the U for collecting the liquid. Pumping was continued during the entire transfer process. Both traps were then cooled with dry ice and sealed off. Dissolved gas in each fraction of the isopropyl ether was then determined by the procedure outlined. In this case, if the Toepler pump was to collect the gas, it was expedient to add a small quantity of air (0.05 millimole) to the system. From the residual 50% of the ether, 0.0006 millimole of methane was recovered, and from the distilled 50% of the ether 0.0003 millimole of methane was recovered.

Application to High-Melting Solvent. Pure solvents which have high vapor pressures at their melting points cannot be degassed by this procedure, but, in principle, gases may be recovered from such solvents—e.g., benzene—by placing the solvent in flask F, in which there is another previously degassed solvent of lower boiling point which will form a low-melting mixture with the high-melting solvent in question.

As an example, benezene was distilled from sodium under an argon atmosphere, then degassed by repeated freezing and melting under vacuum. It was frozen five times; after the second time no gas evolution was noted during the freezing process. As a final step, about 20% of the material was vacuum-distilled and discarded. Some 50 ml. of the residual benzene was added to 50 ml. of degassed n-hexane in apparatus shown in Figure 2 and the noncondensable gases were collected. Dry ice cooling was used in the condenser. Some benzene precipitated in flask F during the operation, but the precipitated benzene was kept fairly well washed down from the condenser by the refluxing liquid. The 0.003 millimole of gas collected was 46% argon, 39.5% nitrogen, and 14.5% oxygen. The high argon content proved the gas was dissolved in the benzene. No tests on recovery of added gases in benzene were made.

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WORK performed under the auspices of the U. S. Atomic Energy Commission.

Convenient Ultramicroburet

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MANY ultramicroburets have been described; the literature has been reviewed by Conway (*1*) and by Kirk (*2*). In general, the better instruments of this kind perform well, but their fragility, difficulties of maintenance, or high cost preclude their use in student or routine clinical laboratories.

The writers have designed a buret, based on the principle of the differential screw, which can be built in any shop equipped with a metal-working lathe and a milling attachment. It is simple, convenient, rugged, and inexpensive as regards cost of materials. The reagent being measured comes in contact with neither stopcock grease nor mercury, and the tube can readily be rinsed when reagents are to be changed.

Figure 1 is an "exploded" view of the drive mechanism.

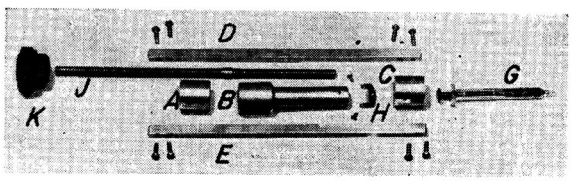

Figure 1. Exploded photograph of drive mechanism for differential-screw ultramicroburet

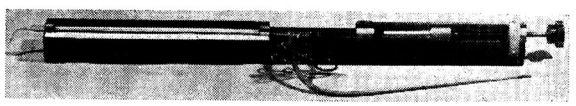

Figure 2. Assembled ultramicroburet using differential screw mechanism

Parts A, B, and C are made from a single piece of $^3/_4$-inch brass rod, in which, before the three parts are cut apart, two slots, $^1/_4$ inch wide and about $^1/_8$ inch deep, are milled at the ends of a diameter of the cross section. These receive guides D and E, which are made from $^1/_4$-inch square steel key stock. A, B, and C are cut apart, and C is drilled (while held in a collet) to hold the barrel of syringe G (a $^1/_4$-ml. tuberculin syringe). A is similarly drilled and tapped for a $^1/_4$-inch screw, 20 threads per inch. B is turned down for part of its length and drilled (while held in a collet) with a tap drill for a $^1/_4$-inch screw, 28 threads per inch. The hole is tapped to a depth of $^3/_4$ inch from the left end of B, as shown, and the remainder of the hole is drilled to clear the screw threads. A small recess, into which the head of the piston of G will fit, is then turned in the right end of B, and stirrup H (made of sheet brass) and two screws are provided to hold the piston to B. A and C are fastened between the guides by screws, as shown; B slides between the guides.

Screw J is threaded on one end to fit A (20 threads per inch), and on the other end to fit B (28 threads per inch). Knob K is provided to turn J.

The barrel of *G* is inserted into *C* from left to right, and the rest of the components are assembled. When all screws are tightened, *G* is cemented in place with sealing wax. The syringe is lubricated and sealed with stopcock grease.

The assembled buret is shown in Figure 2.

A groove is cut in the baseboard to receive one of the guides, and the drive mechanism and buret are secured to the board by brass strips and screws. The buret proper is a capillary provided with a side arm and delivery tip. The end of the capillary which is to fit the syringe is slightly flared with a torch and flaring tool, and the hemispherical end of a 1/8-inch brass rod is pressed into the hot flared tube to form a cavity. The cavity is ground, using Carborundum and the brass rod as a tool. The end of the syringe is ground approximately to fit the cavity, and the glass surfaces are then ground together with Carborundum. Capillary and syringe are cemented together with sealing wax. The rubber tube attached to the side arm makes it easy to rinse the buret, so that titrants may readily be changed. (A pinch or screw clamp closes the rubber tube while titrations are in progress.)

The whole buret assembly is mounted to slide parallel to the bore of the capillary tube, so that the tip of the buret can be moved over or away from a "titration table" (*2*).

The capillaries of the burets are 0.6 mm. in cross section; one revolution of the screw advances the meniscus 3.5 mm., and the position of the meniscus can easily be controlled as precisely as it can be read (0.1 mm., or 0.06 μl.). More delicate control could be attained by using smaller differences of pitch in the differential screw mechanism—i.e., if pitches of 20 and 24 threads per inch were used, the precision of control would be doubled.

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Multiple Spray Technique for Locating Sugars and Polyols on the Same Chromatogram

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Agricultural Research Service,
U. S. Department of Agriculture, New Orleans 19, La.

BY MULTIPLE spraying it is possible to develop spots of sugars and polyols on the same chromatogram. By spraying first with modified Fleury reagent (*1*), followed by *p*-anisidine phosphate (*4*), the characteristic orange spot for inositol is retained as well as the characteristic colors of the sugars reported to develop with the second reagent. By prolonged heating after spraying with the modified Fleury reagent it is possible to extend the usefulness of that reagent to the detection of other polyols such as adonitol, glycerol, mannitol, erythritol, sorbitol, and dulcitol. These alcohols appear as various shades of gray or black: Adonitol and erythritol appear first as black spots, followed by glycerol, mannitol, and sorbitol as gray spots. Dulcitol, also a gray spot, appears last. No background color develops with the Fleury reagent and, after *p*-anisidine phosphate has been sprayed over it, a cream or light buff background appears. As with *p*-anisidine phosphate alone, the intensity and the definition of the spots are enhanced under ultraviolet light. It is possible to detect under ultraviolet light an occasional alcohol spot that is not visible under daylight.

Stock reagents for the Fleury test were prepared as outlined (*1*). However, positive results were obtained repeatedly only when the concentrations of the reagents used in the sprays were changed as follows: 7.5% mercuric nitrate–water, 1 to 1, followed by heating for 10 minutes at 90° to 100° C.; 10% barium acetate–glacial acetic acid, 1 to 10, followed by heating for 10 minutes to develop the inositol spot or 30 minutes to develop other polyols. If polyols other than inositol are known to be present or suspected, it is advisable, to spray a second time with the barium acetate–glacial acetic acid mixture and heat once more for 10 to 30 minutes to bring out the spots. *p*-Anisidine phosphate may be sprayed over the paper only after the final spraying with barium acetate. The *p*-anisidine phosphate is applied as outlined by Mukherjee and Srivastava (*4*).

Sensitivity tests were conducted, and observations made under daylight and ultraviolet light are outlined in Table I, along with the colors observed at each stage in the development of the spots. The unirrigated papers were spotted with an ultramicropipet. Spots were spread over a larger area by superimposing 1 droplet of distilled water delivered from a capillary pipet. The spots, when developed, covered an area of approximately 1 cm.

The color of the inositol spot deepened with time, while those of the sugars faded slightly but were still readily observed under both daylight and ultraviolet light after 7 weeks. A measure of consistency was observed, in that all the sugars tested containing a fructose unit appeared gold-colored under ultraviolet light. Overheating causes the whole strip to turn black. Repeated spraying with the Fleury reagents makes the paper brittle and causes it to disintegrate readily.

Other sugar-detecting reagents such as resorcinol (*5*) and aniline oxalate (*3*), and the Godin reagent (*2*) for polyols other than inositol were unsuccessful as substitutes for *p*-anisidine phosphate, nor could the Fleury spray be used over these reagents. However, when resorcinol was applied over the Fleury sprays and heated for 5 minutes at 105° C., it was found to enhance the color of the inositol spot. The spot changed from orange to a brilliant rose-orange, so intense in color that as little as 1 γ of inositol could be detected.

The multiple spray technique outlined has been applied satisfactorily to chromatographically separated sugars and polyols extracted from plant materials.

Table I. Color Reactions of 20 Sugars and Polyols with Modified Fleury Reagent

Compound	Modified Fleury Reagent Heated after Last Spray		*p*-Anisidine Phosphate (*4*) over Fleury Reagent		Sensitivity, γ	
	10 min.	30 min.	Daylight	Ultraviolet	Daylight	Ultraviolet
Inositol	Orange	Orange	Orange	Red	2	2
Adonitol	Gray	Black	...	Lavender	..[a]	10
Glycerol	...	Gray	White	Lavender	..[a]	10
Mannitol	...	Gray	Dull yellow	Yellow	10	5
Erythritol	Gray	Black	Lavender	Purple	10	10
Sorbitol	...	Gray	Tan	Light brown	20	25
Dulcitol	...	Gray	Tan	Yellow	25	20
Arabinose	...	Tan	Brown	Brown	10	10
Fructose	Yellow	Tan	Golden brown	Gold	10	10
Glucose	...	Black	Dull yellow	Dull yellow	10	10
Mannose	...	Pale tan	Pale tan	Yellow	10	10
Sucrose	Yellow	Brown	Gold	Gold	10	10
Maltose	...	Pale tan	Dull yellow	Dull yellow	10	10
Lactose	...	Pale tan	Dull yellow	Bright yellow	10	10
Melibiose	...	...	Dull yellow	Yellow	5	20
Cellobiose	...	Pale tan	Bright yellow	Bright yellow	20	20
Trehalose	...	...	Pale pink[b]	White	?	10
Raffinose	Yellow	Tan	Tan	Gold	10	20
Melezitose	...	Pale yellow	Tan	Gold	10	10
Glucosamine	Yellow	Yellow	Yellow	White	10	10

[a] Spots not visible in daylight.
[b] Fades rapidly.

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