ANALYTICAL CHEMISTRY

WALTER J. MURPHY, Editorial Director

The 1955 Edition of Reagent Chemicals

THE mechanic is only as competent as his tools are good. Similarly, the accuracy of results reported by the chemical analyst is completely dependent upon the quality of the reagents employed. This is why the work of one of the oldest committees of the AMERICAN CHEMICAL SOCIETY, the Committee on Analytical Reagents, is of such paramount importance to the chemical profession and to all industry, where analytical chemistry is essential in research and in the maintenance of quality.

It is with deep pleasure and pride that we announce the availability of the latest book—"Reagent Chemicals"—the AMERICAN CHEMICAL SOCIETY Specifications made official by action of the Society's Council on April 3, 1955. We of the editorial staff of ANALYTICAL CHEMISTRY are pleased to have been privileged to participate in its preparation. Our part has been to edit and produce it, after the able and hard-working committee turned over to us the completed manuscript. We are particularly proud of our connections with the 1950 and 1955 editions. In contents and in appearance, we believe they are worthy of the official endorsement given to them by the world's largest scientific society.

The preface to the 1950 edition contains some early history that is worth summarizing. As early as 1903, the ACS was concerned with the uncertain quality of chemical reagents then being marketed. In that year, the Council appointed a Committee on the Purity of Chemical Reagents, consisting of John H. Long, chairman; W. F. Hillebrand, secretary; Charles Baskerville, L. M. Dennis, and H. P. Talbot.

This committee held one meeting and outlined a program of specifications. It did not, however, issue any specifications. Nevertheless, the fact that such a committee was in existence did exert influence toward better quality of reagents and a decrease in the discrepancies between labels and contents of packages of reagent chemicals. Unfortunately, the committee was not reappointed when its appointment expired at the spring meeting of the Society in New Orleans in 1915.

One man, a real crusader, continued to emphasize the need for better reagents more accurately labeled. He was W. F. Hillebrand, and through his influence a new group was appointed in January 1917, this time with the official designation Committee on Analyzed Reagents. Hillebrand was appointed chairman, with Charles Baskerville and W. D. Bigelow as the other members.

Space does not permit us to trace the history of this committee from 1917 to the present. The 1950 and now the 1955 editions of "Reagent Chemicals," however, amply testify to the effectiveness of its work. The standards that have been set by the committee in these volumes enjoy world wide recognition because they offer a trustworthy basis of understanding between producer and consumer concerning the quality of laboratory chemicals.

There is one aspect of the committee's history that, perhaps, has not been pointed out. It is the role played by the National Bureau of Standards in helping to improve the quality and labeling of reagent chemicals down through the years.

Hillebrand, who did the early pioneering, is one of the great names in the history of the bureau and of the Society. His name is perpetuated by the Hillebrand Prize given each year by the Chemical Society of Washington (the Washington Section of the ACS). Great credit for the continued success of the committee should go also to W. D. Collins, for years associated with the Geological Survey, who served as chairman in the critical years 1925 to 1942.

Since 1943, the chairman has been Edward Wichers of the National Bureau of Standards. Under his direction, the work of the committee has expanded considerably, making possible the greatly enlarged 1950 and 1955 editions. The office of chairman of a committee, such as that on Reagent Chemicals, requires a tremendous amount of painstaking care, drive, and initiative, and is very time-consuming, yet frequently these services are not fully understood nor appreciated even by those who directly benefit. It is, therefore, very fitting that Edward Wichers be highly commended at this time for his services to the broad field of analysis.

We can understand his reluctance to continue as chairman after 13 or 14 years of intense effort. He has heavy administrative responsibilities at the bureau, and is chairman of the Section on Inorganic Chemistry of the International Union of Pure and Applied Chemistry. While Dr. Wichers has stepped down as chairman of the Committee on Analytical Reagents, he has consented to continue as a member. The analytical profession and industry in general owe him a great debt of gratitude. W. Stanley Clabaugh, also of the Bureau of Standards, has succeeded Dr. Wichers as chairman of the committee.

And now some specific comments on the 1955 edition. It contains 17 new specifications and several hundred changes in requirements and in test procedures. As a reference book, it should be in every laboratory where chemical analyses are made. Despite ever-rising production costs, the 1955 edition is priced at only a very modest increase over the 1950 edition. It is available at $6.50 per copy through the Special Publications Department of the Society.

GAS CHROMATOGRAPHY

Apparatus Requirements for Quantitative Application of Gas-Liquid Partition Chromatography

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Although the technique of gas-liquid partition chromatography was introduced only recently by James and Martin, it already has been widely used for sensitive analysis of a quantitative nature. In this paper, features of apparatus and method are discussed which are important in reproducible analyses of this type. Apparatus features include a bypass sample system which provides for reproducible dosage of samples of known weight, coiled copper-tube columns for convenient thermal control of long columns, and a solid support which gives better separation and is easier to pack into the columns than the commonly used Celite carrier. With the controllable sample injection system used in these experiments, the height of the recorded peaks is sensitive to column temperature, but not to the flow rate of the carrier gas. Conversely, the area under the peak is sensitive to flow rate, but not to column temperature. The degree of flow and temperature control required to make these peak parameters useful for quantitative analysis is discussed.

THE appearance of an ever-increasing number of publications (*1–12, 14–18*) is evidence of the success of the technique for analytical separations known as gas-liquid partition chromatography, which was introduced recently by James and Martin (*9*). Most of these papers have stressed the sensitivity of this technique, and they have shown its application to a variety of systems, but the factors involved in quantitative work of this kind have not yet found detailed treatment. In this and companion papers (*4, 6, 16*) features of apparatus and method are discussed which are essential in developing a reproducible and generally applicable basis for quantitative work involving this technique. The apparatus described was designed with this purpose in mind and is relatively easy to construct and operate.

APPARATUS

The flow scheme of the apparatus used in this study is shown in Figure 1.

The sample to be analyzed is vaporized in the injection chamber, *A*, which is bypassed to permit establishment of thermal equilibrium. The carrier gas flows through a pressure regulator into the preheater coil and through the reference cell of the thermal conductivity detector. From there it passes through the coiled column packed with a granular solid which has been impregnated with a suitable high-boiling liquid. After leaving the column, the carrier gas passes through the measuring cell of the detector and thence either through the flowmeter or the sample collecting traps or to the atmosphere.

The two cells of the thermal conductivity detector are part of a Wheatstone bridge (Figure 2), whose output is recorded on a recording, self-balancing potentiometer. With carrier gas passing through both cells of the detector, the Wheatstone bridge is adjusted so that the recorder traces a zero or near-zero input signal. After thermal equilibrium has been established in the sample-injection chamber, valves 1 and 2 (Figure 1) are turned so that the gas from the reference cell passes through the chamber containing the vaporized sample and sweeps the vapors into the column. Components of the mixture travel at different rates corresponding to their activities in the particular solvent and emerge separately, while pure carrier gas issues between components. The presence of sample vapor in the measuring cell causes an increase in the wire temperature, which is proportional to the concentration of vapor. The increase in temperature results in an unbalance of the Wheatstone bridge, which is recorded by the potentiometer over a period of time as a peak. When pure carrier gas flows through the cell again, the wire returns to its original temperature and the recorder returns to the original base line.

A record of a typical analysis is shown in Figure 3, where each peak represents an individual component of the sample recording its separate emergence from the column. As shown by James and Martin (*9*) and others, the time of emergence of the components can be used to identify them. The sample composition is estimated quantitatively on the basis of various peak param-

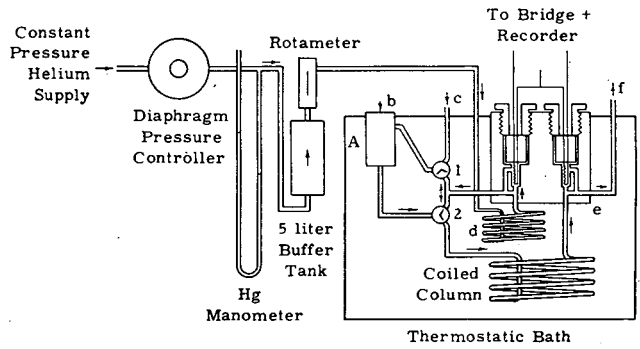

Figure 1. Schematic diagram of typical gas-liquid partition chromatography apparatus

b. **Bypass sample-injection chamber**
c. **Vacuum**
d. **Helium preheater**
e. **Thermal conductivity detector**
f. **To flow-measuring device or cold traps**

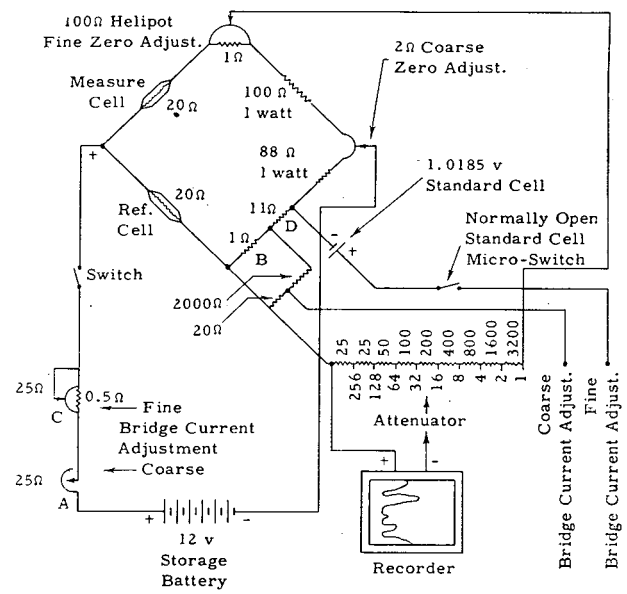

Figure 2. Schematic wiring diagram for 12-volt 200-ma. bridge for thermal conductivity detector

eters as discussed later. The phases of apparatus design and operating procedure which may affect these peak parameters have been studied and are discussed separately in the following sections.

Sample-Injection Systems. The manner in which the sample is introduced onto the column is one of the most important factors influencing the precision of analysis by gas-liquid partition chromatography. An often used method is to inject liquid samples directly into the carrier gas stream just ahead of the column packing by means of a hypodermic syringe and a serum cap. This method does not permit controllable sample dosage, and results in a sample chamber of indefinite volume. It has been shown (*16*) that with a sample chamber of fixed volume, the emergence time and peak parameters reduced to unit weight are independent of sample weight, but in the case of direct injection of liquid sample these quantities become functions of the sample weight. The latter condition makes it necessary to calibrate the apparatus over the range of sample weights to be used, and complicates the development of generally applicable constants from gas-liquid partition chromatographic measurements. These considerations led to the development of the bypass sample-injection system shown in Figure 4.

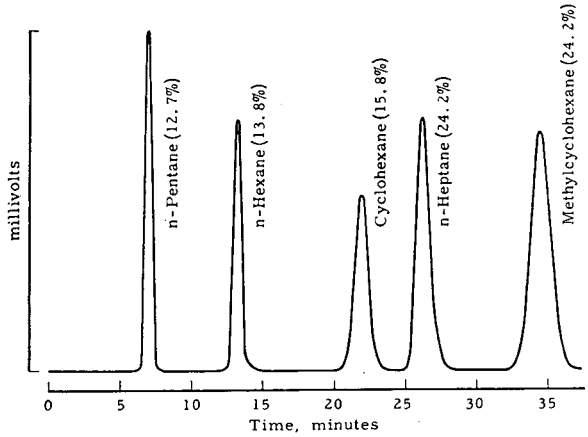

Figure 3. Chromatogram of hydrocarbon mixture

The sample is introduced into chamber *A*, where it is completely vaporized and brought to column operating temperature while the chamber is isolated from the column by a system of valves. The valves are quick-acting Hoke stainless steel diaphragm valves. Two of the valves are modified by drilling into the base as shown in Figure 4. In this way, the dead-end space in the system containing sample vapors is practically eliminated. The chamber shown in Figure 4 is equipped with a bellows crusher, so that weighed samples in glass ampoules can be introduced and crushed after isolating the chamber. When relatively large samples are being used, the chamber can be evacuated through valve 1 prior to crushing the ampoule, so that the final pressure in the chamber will not exceed the saturation pressure of the sample at the operating temperature. In cases where the column liquid is sensitive to air, the chamber may be back-purged with helium through valves 4 and 1 before the ampoule is crushed.

The same valve system can be used with a variety of sample chambers. A chamber equipped for injection by hypodermic syringe can be used in place of the bellows crusher. The valves also can be used with a gas-handling system for samples which are normally gaseous.

The bypass sample system permits introduction of the sample into the column in an exactly reproducible manner, if the total pressure does not exceed the column operating pressure and the sample partial pressure does not exceed its saturation pressure in the chamber. The reproducibility of sample injection leads to peak heights and peak widths per unit weight of sample that are constant over a wide range of sample size. This makes it unnecessary to calibrate the apparatus with respect to sample size. One calibration then suffices for all sample sizes below that at which the partial pressure in the sample chamber exceeds saturation pressure.

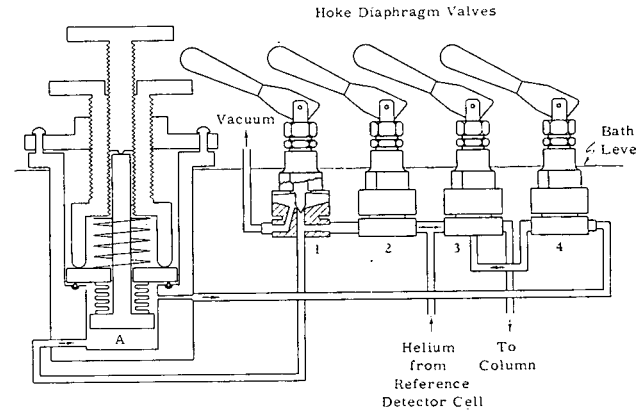

Figure 4. Bypass sample-injection system with bellows crusher

It is shown elsewhere (*16*) that the peak width increases in proportion to chamber volume. As this results in a decrease in component resolution, it is desirable to keep the chamber volume at a minimum consistent with other requirements. The chamber volume found useful with the apparatus described was 10 ml.

Columns and Column Packing. The best quantitative estimation of sample composition can be made when all of the peaks are completely separated. Adequate separation of substances having nearly the same activities often requires the use of long columns. Columns 50 feet or longer can be conveniently made from copper tubing, which can be coiled after packing to fit into relatively small constant temperature baths. The metal columns are easy to pack because they can be vibrated vigorously without danger of breakage. Furthermore, the columns can be attached to the rest of the apparatus with copper tubing fittings; Swagelok or Ermeto fittings are the preferred types.

Fifty-foot columns of this type have been used in the separation of C_1-C_5 hydrocarbons and C_5-C_6 paraffins (*4*, *6*).

Columns made from copper tubing $^1/_4$ or $^3/_8$ inch in outside diameter are generally used for quantitative analysis. Often it becomes necessary to identify peaks in the chromatogram by infrared or mass-spectrometric methods. These small-diameter columns will generally furnish enough sample for mass-spectrometric identification. However, if the component to be identified is a minor constituent of the sample or if infrared or other methods of identification are to be used, it is necessary to use columns of larger diameter. A column 4.2 cm. in inside diameter and 295 cm. long has been found useful for this purpose (*5*). The size of the sample that can be separated on this column depends on the type of sample and on the column parameters. For samples containing approximately equal amounts of two components differing by two carbon atoms, the column will separate the components of a 10-gram sample without serious overlap of peaks. Unlike liquid chromatographic columns, the gas-liquid partition chromatography columns do not seriously lose efficiency when the diameter of the column is increased sevenfold. This is illustrated in Figure 5, where the efficiency of a 0.27-inch (7 mm. in inside diameter) column can be compared to that of a 1.71-inch (4.2 cm. in inside diameter) column. The columns were operated at the same temperature and with sample size and carrier gas flows which were equal per unit cross section. In this case, less than a 50% increase in column length almost completely compensates for the loss in efficiency caused by a 600% increase in column diameter.

Solid Supports for Immobile Liquid Phase. The use of ground diatomaceous earth (Celite) in long columns of small diameter has certain disadvantages. Celite that has been coated with the stationary liquid tends to form clusters which do not flow freely and tend to pack into an impermeable mass; this makes loading of long columns a difficult and time-consuming task. Moreover, the pressure drop characteristic of this carrier is high—for instance, a 50-foot column of 1/4-inch diameter packed with Celite would require a pressure of nearly 100 pounds per square inch to establish the required flow. In the work described, a solid support has been used which does not have these disadvantages, and also displays a higher resolving power per unit length than Celite under comparable conditions. This support is a crushed diatomaceous earth firebrick (Johns-Manville C-22), which flows freely even when coated with 40 grams of liquid per 100 grams of solid; this property greatly facilitates uniform packing of the columns. A firebrick, which has comparable properties and is marketed in Germany under the name of Sterchamol, has been successfully used for this purpose by Keulemans and Kwantes (*12*).

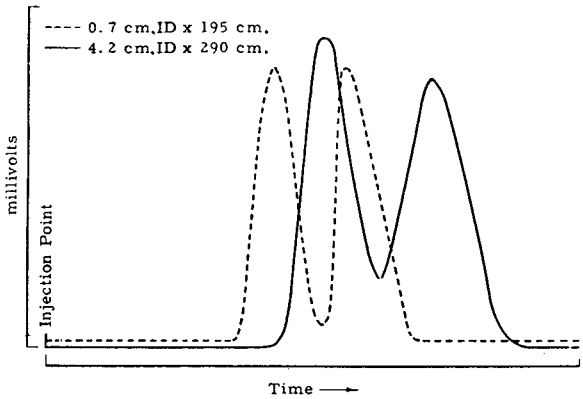

Figure 5. Separations with different column diameters

The differences in resolving power between the two materials (Celite and C-22 firebrick) is illustrated in Figure 6. The curve shown in the dotted line was obtained on a 6-foot column, 1/4 inch in outside diameter, packed with Celite 545 impregnated with 40 grams of diisodecyl phthalate per 100 grams of solid. The curve shown in the solid line was obtained on a column of the same length, packed with 60- to 80-mesh C-22 firebrick impregnated with the same amount of phthalate. The components of the test mixture, which consisted of 2,3-dimethylbutane (49%) and 3-methylpentane (51%), had nearly identical retention volumes (*6*) in the two columns. The columns were operated at identical flow rates and temperatures. Under these conditions the depth of the valley between peaks provides a basis for direct comparison of the resolving power of the columns. The deep valley between the peaks (Figure 6) for the C-22 firebrick column graphically shows the greater resolving power of columns packed with the firebrick. Further evidence of the superiority of firebrick was provided by other experiments, in which it was found that under comparable conditions Celite columns provided three to four theoretical plates per centimeter of column length, whereas C-22 firebrick gave five to six plates per centimeter calculated by a method equivalent to method II of James and Martin (*9*).

Since the small particle size of Celite (passes 100-mesh) is chiefly responsible for both the high pressure drop and the difficulty in packing columns of small diameter, the effects of particle size on the resolution and pressure drop were studied. A knowledge of these effects permits selection of a solid support of the proper particle size for a column of given length, in order to obtain the best separation with a pressure drop within the practical range of existing apparatus. Figure 7 indicates that the pressure drop through a column rises sharply when it is packed with materials finer than about 50-mesh (292-micron particle diameter), whereas Figure 8 indicates that with particles larger than 100-mesh (147-micron), the resolution is nearly a linear function of the particle diameter. From these data it would appear that the firebrick should be screened to include only 35- to 60-mesh material for optimum resolution with a minimum pressure drop.

The crushed firebrick has been used as the solid support for separations involving alcohols, hydrocarbons, ketones, aldehydes, and chlorides and in each case the firebrick was found to be superior to the Celite formerly used.

Preparation of Column Packing. In order to ensure an even distribution of the stationary liquid phase over the surface of the solid, the liquid [aspects of the selection of the proper liquid phase are discussed in another paper (*16*)] is dissolved in a low boiling solvent and made into a loose slurry with the solid support. Thoroughly dried solid is added in the ratio of 100 grams of solid to 40 grams of stationary liquid, and the mixture is stirred thoroughly. The low boiling solvent is removed on a steam bath while the mixture is stirred continuously. The final traces of low boiling solvent are removed in an explosion proof oven at about 105° C. Firebrick packing prepared in this manner packs to a bulk density of about 0.63 grams per ml. in the column.

Detector Design. A detector for quantitative analysis by gas-liquid partition chromatography methods should have the following characteristics:

1. Equal response for all sample components in the carrier gas stream
2. Linear response with concentration in the range considered
3. Insensitivity to flow rate and pressure changes
4. High signal to noise ratio

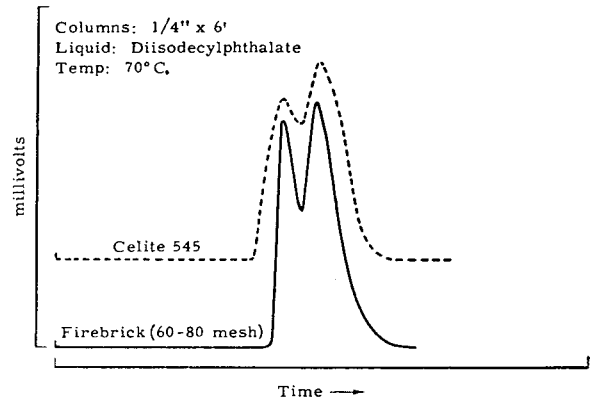

Figure 6. Relative resolving power of Celite and firebrick

Table I. Thermal Conductivity of Common Liquids and Carrier Gases Determined in Their Vapor State at 200° F.[a]

Material	Thermal Conductivity, (B.T.U./Hour, ° F., Feet) × 10^{-3}
n-Pentane	12.5
n-Hexane	12.0
n-Heptane	11.6
n-Octane	11.2
n-Nonane	10.7
Ethyl alcohol	11.7
Methanol	11.4
Acetaldehyde	10.2
Acetone	9.9
Cyclohexane	10.4
Benzene	9.1
Toluene	11.5
Xylene	9.8
Helium	97.7
Nitrogen	17.7

[a] (*13*).

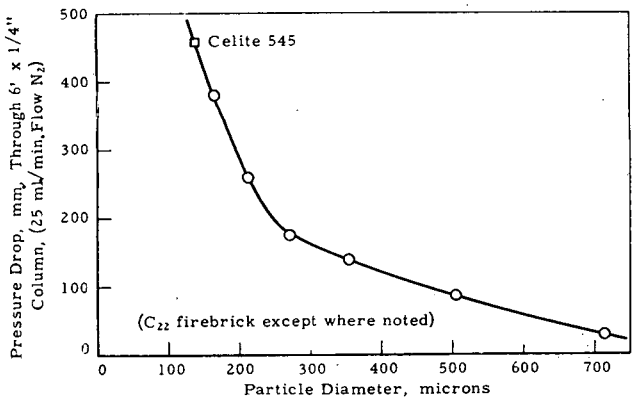

Figure 7. Effect of particle size on pressure drop

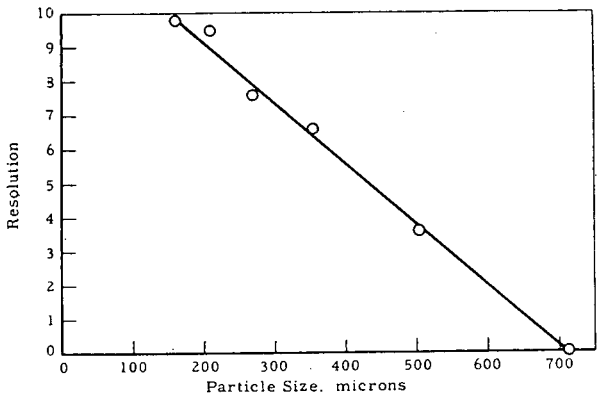

Figure 8. Effect of particle size on resolution

Resolution expressed as ratio of peak height to height of saddle between peaks under comparable conditions. See Figure 6

5. Rapid response
6. Low volume
7. Long-term stability

Several types of detectors have been used, including automatic titrators (*9*), gas density balances (*10*), β-ray ionization gages, surface potential detectors (*7*), and thermal conductivity cells (*7*, *17*). Thermal conductivity detectors are widely used because they are simple to build and to maintain and, properly designed, satisfactorily approach these ideal conditions. Several designs of this type of sensing device are described in this paper.

When thermal conductivity detectors are used, helium (welding grade, 99% pure) is advantageously used as the carrier gas because of its high thermal conductivity (Table I). As it is an order of magnitude higher than most compounds normally subjected to gas-liquid partition chromatographic analysis, the difference in the thermal conductivities of helium and any of these compounds is relatively constant. Consequently, the response of the compounds recorded in the thermograms is nearly the same for similar concentrations. This gives helium an advantage over commonly used carrier gases, which have much lower thermal conductivities. Hydrogen has a higher thermal conductivity, but is not commonly used because of its reactivity. Values for thermal conductivity of a few compounds of interest (Table I) show that the greatest error possible due to the assumption that all these organic compounds have equal thermal conductivities is approximately 3.5%. In many cases the thermal conductivity of gas mixtures is not linear in concentration and may cause larger errors.

The signal to noise ratio, or the usable sensitivity of a thermal conductivity detector, is dependent upon many factors, including the resistance and temperature coefficient of the wires and the configuration of the Wheatstone bridge, of which the detector is a part. The approach to linearity of the response with concentration is also a function of the bridge configuration as well as of the linearity of the temperature coefficient of the thermal conductivity cell wire. The wire from which the thermal conductivity detector is made should have as high a resistance as practical and should have a high linear temperature coefficient of resistance. As the Wheatstone bridge used to measure the resistance of the thermal conductivity wire has considerable effect upon the sensitivity and linearity of the detector, an analysis of the various possible bridge arrangements has been made. Standard practice in Wheatstone bridge design is to have all four arms of the bridge of equal resistance, as shown in Figure 9*a*. If four separate wires are used in the detector (two reference cell wires and two measuring cell wires), it is common practice to use the four wires as the four arms of the bridge as in Figure 9*c*. However, when the bridge unbalance is recorded on a recording potentiometer, as is done in gas-liquid partition chromatography, these equal-arm bridges do not give the maximum sensitivity or the best possible linearity. As is shown in Table II, where the output signals for the four bridge arrangements of Figure 9 are calculated at two different resistances of the measuring cell wire, greater sensitivity and more linear response can be achieved by wiring the bridges as shown in Figure 9*b*, or *d*. The improved response is achieved by increasing the voltage to the bridge to 12 volts and by using high resistances in the fixed arms of the bridges to fix the current at the maximum permitted by the thermal conductivity wires. For detectors with four wires, the two reference wires are made a part of one arm, the two measuring wires are a part of another arm of the bridge, and two fixed resistors are used as the other two arms. With this arrangement, the change in current in the measuring cell wire as the sample vapors pass over the wire is at a minimum, which accounts for the improved signal.

Near atmospheric pressure the thermal conductivity of a gas

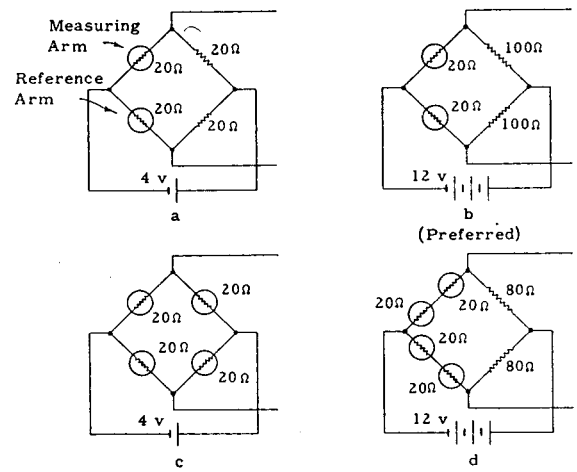

Figure 9. Wheatstone bridges for two- and four-cell thermal conductivity detectors

Table II. Calculated Signal from Various Thermal Conductivity Detectors[a]

Bridge Type (Figure 9)	S_1[b], Signal for 0.01-Ohm Increase, Mv.	S_2, Signal for 1.0-Ohm Increase, Mv.	% Signal Increase over *a*	% Nonlinearity, $\frac{(100\ S_1 - S_2)}{S_2} \times 100$
a	0.4998	48.78	...	2.4
b	0.8333	82.64	69.4	0.8
c	0.9997	97.56	100.0	2.5
d	1.6669	163.93	236.1	1.7

[a] Bridge current 200 ma. at balance in all cases.
[b] Increase in measuring wire resistance; reference wire remains constant.

does not change appreciably with pressure and consequently the thermal conductivity detector is not inherently pressure-sensitive. The influence of pressure cannot be neglected, however, because the concentration of components in the effluent is sensitive to pressure.

The temperature of the sensing wire varies with the rate of flow as well as with the thermal conductivity of the gas passing over it. This flow sensitivity imposes the need of careful control of the flow of the carrier gas. Alternatively, the cell block in which the sensing wire is placed may be designed to minimize the effect of fluctuations in the gas flow. The sensing wires used in this study were made by the Gow-Mac Instrument Co. The heated elements have a 15-ohm (cold resistance) coiled wire mounted on leads which are embedded in plastic by the manufacturer for easy mounting in the cell block. Three cell block designs have been used with different flow patterns and, consequently, different flow sensitivities and time constants. The convection-diffusion type cell (Figure 10, *B*) has practically zero flow sensitivity but has a 20-second time constant (time required for 60% response). This relatively long time constant causes some distortion of peaks when the retention time is relatively small. The time constant of the flow-through cell, shown in Figure 10, *A*, is limited by the pen speed of the recorder (2 seconds full scale), but its flow sensitivity is sufficient to cause some irregularity in the base line at the highest sensitivity setting. The self-purging cell (Figure 10, *C*) represents a compromise; it has a time constant small enough (10 seconds) so that no sensible distortion of peaks is observed and its flow sensitivity is such that operation monitored by commercially available pressure controllers gives a stable base line.

Thermal conductivity detectors are highly sensitive to changes in bridge current. A change of only 0.1% in the bridge current will change the response 0.3%. During the major portion of normal discharge, however, a properly charged lead storage battery in good condition will furnish current of adequate stability. Other direct current supplies may be used if they are designed for stability and are choked sufficiently so as not to reduce the sensitivity of the 1-mv. Brown recorder by persistence of alternating current ripple.

The schematic wiring diagram for a two-cell thermal conductivity detector (one for reference, one for measuring) is given in Figure 2.

Coarse bridge current adjustment is made on the 25-ohm variable resistor, *A*, while the recorder reads the voltage drop across the 1-ohm resistor, *B*. Final adjustment of the bridge current is made with resistor *C*, by use of voltage difference between the standard cell and the voltage drop across the 12 ohms in resistors *B* and *D* as a highly sensitive indication of the proper bridge current. A 0.1% change in bridge current will produce a 1-mv. signal from this arrangement, which can be read on the recorder. The current-measuring resistors are placed in the reference arm of the bridge, so that current can be set exactly, even though the bridge is not balanced.

Detector Sensitivity. Frequently the sensitivity of the sensing unit is expressed in terms of the weight of sample required to produce full scale deflection. This value is a function of the voltage sensitivity of the recorder, the retention volume of the sample, and column conditions during the experiment. This makes direct comparison of sensitivity values impossible and points to the need of an expression which can provide a basis of comparison. A peak area term reduced to a standard basis by taking into account the factors mentioned above can be made to represent detector sensitivity in a generally applicable manner as follows:

$$S\,(\text{ml.} \times \text{mv. per mg.}) = \frac{A \times C_1 \times C_2 \times C_3}{W}$$

where

S = sensitivity parameter
A = peak area, sq. cm.
C_1 = recorder sensitivity, mv. per cm. of chart
C_2 = chart speed, minutes per cm.
C_3 = flow rate at exit of column, ml. per minute corrected to column temperature and atmospheric pressure
W = weight of sample introduced at head of the column, mg.

Many detectors have high sensitivity but a great deal of background noise; consequently the usable sensitivity is reduced by the noise level. Therefore, any report of detector sensitivity should include the background noise level, preferably expressed as millivolts.

A thermal conductivity detector with the flow pattern shown in Figure 10, *C*, and the Wheatstone bridge shown in Figure 2 gives a sensitivity of about 312 ml. × mv. per mg. with a background noise level of about 0.005 mv. For materials emerging in approximately 15 minutes the sensing system described, operating at maximum sensitivity, will give full scale deflection (1 mv.) for about 0.1 mg. of sample.

Temperature Control. James and Phillips (*11*) have reported that a 1° C. change in column operating temperature results in a 10% change in retention volume. The temperature at which this was measured was not stated, but appears to be about 50° C. Work done in this laboratory (*16*) has shown that a 1° C. change in operating temperature at 105° C. results in an average change of 4% in retention volume. As a change in retention volume will bring about a change in peak height, which is commonly used for quantitative estimations, the effect of temperature change on peak height was determined experimentally. The results of a few of these tests are shown in Table III. A mixture of five saturated hydrocarbons was analyzed in this experiment; a 10-foot column packed with a high-boiling saturated hydrocarbon (squalane) supported on C-22 firebrick was used in order to secure maximum linearity of the isotherms. A bypass sample injection

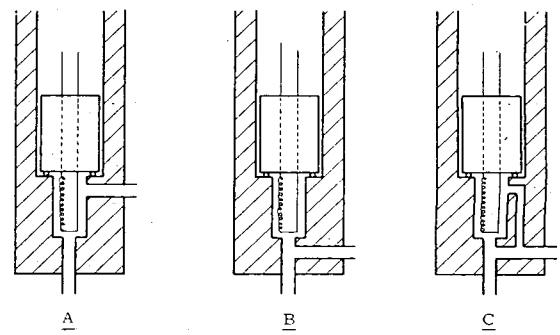

Figure 10. Thermal conductivity cell designs

A. Flow through cell
Time constant. 1 second
Flow sensitivity. 1 μv. per ml. per minute change in one cell

B. Convection-diffusion cell
Time constant. 20 seconds
Insensitive to flow changes

C. Self-purging cell
Time constant. 10 seconds
Insensitive to flow changes

Table III. Effect of Temperature on Adjusted Peak Height and Retention Volume

(10 foot × 1/4 inch squalane on firebrick. Column, 40 ml. per minute helium flow rate)

Sample Component[a]	V_R[b] at 80° C.	V_R at 105° C.	% Change per ° C. at 80° C.	P.H./Mg., Mv./Mg. At 80° C.	At 105° C.	% Change per ° C.
n-Pentane	557	379	1.3	4.14	5.74	1.5
n-Hexane	1237	737	1.6	2.24	3.75	2.7
Cyclohexane	2171	1230	1.7	1.21	2.20	3.3
n-Heptane	2821	1476	1.9	1.02	2.06	4.1
Methylcyclohexane	3700	1949	1.9	0.70	1.39	3.9

[a] In order of emergence.
[b] V_R, retention volume. P.H./mg., adjusted peak height.

Table IV. Effect of Temperature on Peak Area

Sample Component[a]	Area, Ml. × Mv./Mg. At 80° C.	Area, Ml. × Mv./Mg. At 105° C.	% Change per ° C.
n-Pentane	134	145	0.3
n-Hexane	132	150	0.5
Cyclohexane	118	133	0.5
n-Heptane	122	141	0.6
Methylcyclohexane	107	124	0.6

Column and conditions same as in Table III.

[a] In order of emergence.

system was used, so that samples could be weighed and the peak parameters—i.e., peak height per mg.—would be independent of sample size. Tests were made at 80° and 105° C. It is seen (Table III) that the change in peak height is greater in all cases than the change in retention volume. A change of 1° C. in operating temperature would result in a 2.4% error in the determination of methylcyclohexane by relative peak height using *n*-pentane as an internal standard (*17*). The error becomes smaller as the retention volumes of the internal standard and the unknown become more nearly equal.

Peak area, as distinguished from peak height, is only slightly affected by temperature changes. If the temperature changes during a run, the partition coefficient will change about 5% per degree. This will cause the concentration of vapor in the eluted gas to drop by a corresponding amount; thus the peak height will fluctuate by about 4% per degree. The band will be eluted in a proportionally larger volume of gas, however, so the area of the peak will not be affected. Some experiments were made to illustrate this point, under conditions similar to the preceding series (Table IV). It is seen that a change of 1° C. in operating temperature would result in only 0.3% error in the determination of methylcyclohexane when *n*-pentane is used as an internal standard.

Constant-Temperature Baths. The use of coiled copper tubing for making gas-liquid partition chromatography columns makes possible the use of relatively small constant-temperature baths even for very long columns.

Where the bypass sample injection system is not used, a 1-gallon Dewar oil bath will accommodate the detector unit and at least 20 feet of 1/4-inch outside-diameter column with enough room left for helium preheater, bath heaters, stirrer, and temperature regulator. Somewhat larger baths are required to accommodate the valves and chamber of the bypass injection system. Where the apparatus is primarily used for research, and where columns of different sizes and characteristics are used, stirred air baths are convenient.

The bath used for much of the work described here was made from two concentric Transite boxes separated by 1.5 inches of rock wool insulation. The inner box is a 16-inch cube. The cover is divided laterally across the center to facilitate its removal. A wide-tongue-and-groove joint is used at this break to decrease heat loss. Stirring of the air is provided by a centrifugal blower 6 inches in diameter and 3 inches wide, located in the center of the rear wall. A 2-inch-wide baffle on the opposite (front wall) directs the air stream through the center of the box into the blower. Three heaters of bare Chromel wire, open coil construction, are supported around the periphery of the blower on four Transite blocks. The quick-heat unit is designed for 1500 watts, the integral control heater for 800 watts, and the on-off heater for 100 watts nominal power rating. The integral control heater is supplied through a motor-driven Powerstat. The position of the Powerstat is automatically adjusted according to the demands (change in ambient temperature or line voltage) until only half of the nominal wattage of the on-off heater is required and its on and off periods are equal. This bath maintains constant air temperature to within ±0.015° C. for temperature ranging from slightly above room temperature to 200° C.

Baths of this type may easily be equipped with refrigeration coils for operation below room temperature. However, because of the low heat capacity of air, considerable time must be allowed for temperature equilibration if the temperature of any part of the system is upset, as, for instance, during sample injection. Another promising heat-conditioning unit, described by Adams, Gernard, and Kimberlin (*1*), makes use of fluidized solids and thus tends to combine the advantage of the more rapid temperature equilibration of the liquid bath with the cleanliness and ease of column replacement of the air bath.

Detector Temperature Control. Unless the two wires in the thermal conductivity detector are equal in resistance, the balance point of the bridge may change with temperature. For this reason, and also to prevent condensation of sample in the detector, the whole detector unit is included in the same constant-temperature bath as the column and sample injection system. The degree of temperature control required for proper column operation is adequate to maintain stability of the zero balance of the detector.

Flow Control and Measurement. Because the retention volume (amount of gas required to carry the individual components of the sample through the column) is used to identify the sample components, it is essential that the flow rate be known, and constant. Similarly important for quantitative estimation is the effect of flow rate on the shape of the emergent peak. The effects of changes in flow rate on peak height and peak area were determined experimentally with the same sample and the same column as used in the previous temperature tests. The results of tests at two flow rates (Table V) indicate that the height of the peak is not sensitive to flow rate. The differences shown in Table V are small and probably reflect weighing errors. The areas of these same peaks are shown in Table VI. The areas calculated in terms of milliliters × millivolts. per milligram are essentially constant with a twofold change in flow rate, except for small differences which again presumably arise from weighing errors. As the area calculated is constant, the area in square centimeters changes in direct proportion to changes in flow rate. The flow rate during an individual experiment consequently must be constant, and repeatable, to within 1% in order to make use of the area of the peak to estimate sample composition to within 1%. For columns with low flow rates (down to 20 ml. per minute) this imposes a more rigid limit on flow rate control than does the flow sensitivity of the detector.

The rate of flow is governed by the pressure drop through the column and is controlled by controlling the pressure at the head of the column. As flow rate is nearly a linear function of pressure (Figure 11), the pressure at the head of the column must be controlled with the accuracy that is desired in the final analysis of the sample. A Moore Nullmatic pressure regulator in series with a standard gas cylinder reducing regulator has been found to give adequate control of column pressure.

Table V. Effect of Flow Rate on Adjusted Peak Height

(Column temp. 80° C., 10-foot 1/4-inch column with squalane on firebrick)

Sample Component	P.H./Mg., Mv./Mg. At 21.25 ml./min.	P.H./Mg., Mv./Mg. At 40.02 ml./min.	% Difference per Ml./Min. Flow change
n-Pentane	4.37	4.14	0.3
n-Hexane	2.24	2.24	0.0
Cyclohexane	1.18	1.21	0.1
n-Heptane	1.03	1.02	0.05
Methylcyclohexane	0.70	0.70	0.0

Table VI. Effect of Flow Rate on Peak Area[a]

(Column temp. 80° C., 10-foot 1/4-inch column with squalane on firebrick)

Sample Component	Area, Ml. × Mv./Mg. At 21.25 ml./min.	Area, Ml. × Mv./Mg. At 40.02 ml./min.	% Difference per Ml./Min. Flow Change
n-Pentane	136	134	−0.08
n-Hexane	130	132	+0.08
Cyclohexane	123	118	−0.2
n-Heptane	130	122	−0.3
Methylcyclohexane	114	117	+0.1

[a] The bridge used in these determinations was a 4-volt bridge (Figure 9*a*) operated at only 75 ma. and was consequently less sensitive than that shown in Figure 2.

Table VII. Accuracy by Various Methods of Area Measurement with and without Previous Calibration

Sample Component	Sample Composition, Wt. %	Measured by Planimeter		Measured by Weight of Paper		Measured by Peak Height × ½ Width			
		Area, sq. cm.	% of total area	Area, sq. cm.	% of total area	Area, sq. cm.	% of total area	Corrected by Calibration Area	Corrected by Calibration %
n-Pentane	12.7	4.4	13.9	4.52	14.5	3.87	13.7	2.71	12.8
n-Hexane	13.8	4.7	14.9	4.68	15.0	4.17	14.7	2.90	13.7
Cyclohexane	15.8	5.1	16.1	4.81	15.4	4.30	15.2	3.31	15.7
n-Heptane	24.2	7.8	24.7	7.81	25.0	7.12	25.2	5.09	24.1
Methylcyclohexane	33.5	9.6	30.4	9.46	30.2	8.82	31.2	7.11	33.7
Standard dev., %			7.8		9.8		6.8		0.76

The chromatographic column acts as a buffer to prevent the small surges from the pressure controller from affecting the measuring cell. The reference cell has no such built-in buffer; a small buffer tank (5-liter) is placed in series between the controller and the reference thermal conductivity cell. A rotameter is also included in this line to give a rough indication of flow rate. Rotameters in general are not accurate enough to be used to reproduce the flow rate with the required precision. The flow rates reported here were measured by collecting the exit gas over water (*18*). Flowmeters which depend on pressure developed across a constriction are not satisfactory for permanent installation at the exit of the column, because the back pressure on the column and detector increases as the sample components emerge and may have deleterious effects on the peak shape and emergence time.

CALCULATION OF SAMPLE COMPOSITION FROM ELUTION CURVES

For computation of sample composition from chromatograms, the heights of the individual peaks have been used, as well as their areas. The dependence of these parameters on temperature and flow rate, discussed in preceding paragraphs, will play a part in making the appropriate choice of parameters for a given analysis. Additional factors in the selection are given below.

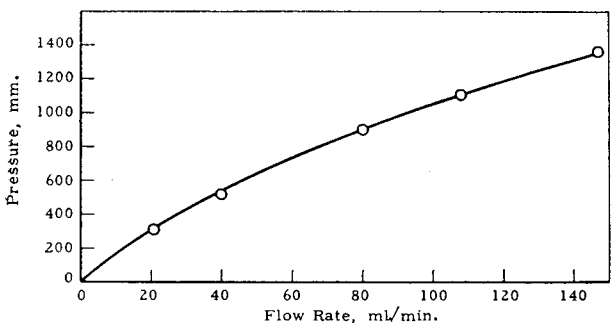

Figure 11. Relation of flow rate to pressure

Use of Peak Heights. As the measurement of peak height is simple, this parameter is used whenever enough samples of similar type are to be analyzed to justify the calibration with pure compounds. The peak height per unit weight of compound is dependent on the efficiency of the column. Since the efficiency in turn is affected by many changes in operating conditions, the reduced peak height is not a suitable parameter for the purpose. It is preferable to use an internal standard—i.e., addition of a known compound in known concentration. Comparison of the peak heights of the peaks to be analyzed with that of the internal standard gives the desired information. The material selected as the internal standard must not be part of the sample to be analyzed, and it must have a retention volume different from any component of the sample. The relative peak height is temperature-dependent (Table III), but the effect of temperature changes can be minimized by use of an internal standard which has a retention volume intermediate among the components of the sample.

Use of Peak Area. Although the measurement of peak area is somewhat more laborious than that of peak height, this parameter has found considerable use. When helium is used as the carrier gas in a thermal conductivity detector, the areas under the peaks can be related to the sample composition with fair accuracy without the prior calibration discussed in the preceding sections. This was demonstrated by experiment, as shown in Table VII, calculated from the chromatogram shown in Figure 3. Here the area of each peak was determined by three different methods; the composition of the mixture is given by the ratios of the individual peak areas to the total area of all the peaks. Whenever this complete analysis is not desired or possible, a known standard substance may be added as before, and the concentration of the unknown found from the ratio of the two corresponding peaks. In this case, an internal standard should be selected with nearly the same thermal conductivity as the component or components to be measured in order to reduce errors arising from that source. The sample composition determined by relative area shows a standard deviation of 6.8 to 9.8%, depending upon how the areas are measured. The deviation is considerably lowered by adjusting the peak areas by means of factors determined by calibrating with the pure compounds corresponding to the peaks (*16*). Application of this procedure to the third method (peak height times width at half height) reduced the standard deviation to 0.76%, as shown in Table VII.

Peak area measurements are especially useful in cases where two or more components of a multicomponent sample are only partially separated. The total area of the doublet or multiplet can be measured and considered as a single component. Alternatively the individual components of the doublet may sometimes be computed, if they are close enough to representing normal distribution curves. Peak height measurements do not give reliable results unless the peaks are separated far enough that one component does not affect another.

Methods of Measuring Area under Elution Curves. The most common and probably the most time-consuming method of integration of the area under a peak involves the use of a polar planimeter. The method suggested by Cremer (*3*) is much faster and easier to use. In this method the product of the height of the peak and the width of the peak at half height is used to represent the area under the curve. When the curves are nearly Gaussian, the method of Cremer gives results that correspond to 0.84 times the area found by integration. Probably the most sensitive method is furnished by cutting out the peaks and weighing the paper on an analytical balance. The method is not used extensively, because its accuracy depends upon the uniformity and the moisture content of the paper as well as the skill of the operator in cutting the paper. Furthermore, the method is time-consuming and the record is destroyed in making the measurement. The most convenient, if not the most accurate, method is available in the use of mechanical or electrical integrators which can be attached directly to the recorder. One such instrument is the ball and disk integrator manufactured by the Librascope Co. These integrators are accurate to 0.5% or better.

SUMMARY AND CONCLUSIONS

Quantitative estimates of the composition of many mixtures of organic compounds can be made by using the height or the area of the peaks resulting from gas-liquid partition chromatographic analysis. The peak height has been shown to be virtually insensitive to flow rate, but sensitive to fluctuations of column operating temperature. The reverse is true of peak areas. Hence, if peak heights are to be used for calculations, very close temperature control is required, whereas poor control can be tolerated when areas are utilized. If peak heights are to be used, relatively poor gas pressure regulation can be tolerated, whereas for area measurements very close control is needed.

A bypass sample-injection system is suggested in order to eliminate the effects of variation in sample size and to increase reproducibility.

An inert carrier material, C-22 firebrick, is recommended in place of the commonly used Celite. The crushed firebrick offers easier packing, lower pressure drop, and improved resolution, which makes it a highly suitable inert packing material, especially for long columns.

A thermal conductivity detector features ease of construction, high sensitivity, stability, and a low time constant. Helium is recommended as the carrier gas when thermal conductivity detectors are used, because of the high sensitivity and low noise level. Furthermore, the recorded response to all compounds in the usual range of analysis is nearly the same on a unit weight basis, and proportional to the concentration of the compounds.

Coiled copper tube columns are easily constructed and make possible convenient thermal control of long columns.

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GAS CHROMATOGRAPHY

Analysis of Gaseous Hydrocarbons by Gas-Liquid Partition Chromatography

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A study has been made of the applicability of gas-liquid partition chromatography to the quantitative analysis of complex mixtures of hydrocarbon gases. The results indicate that good resolution of most of the hydrocarbons through C_5 can be achieved with a two-stage column in which the sample is partitioned first by diisodecylphthalate and then by dimethylsulfolane. Use of a 50-foot column containing only dimethylsulfolane separates isobutylene from 1-butene and gives excellent resolution of the pentenes. Helium is used as the carrier gas and the column effluent is monitored by continuously recording its thermal conductivity. Recorded curves for several hydrocarbon mixtures illustrate the separations that are achieved. Qualitatively, the method permits the separation and identification of all the commonly encountered hydrocarbon gases. A good quantitative measure of composition is obtained from the distribution of area under the peaks of the recorded curves.

THE technique of gas-liquid partition chromatography for the separation and analysis of complex mixtures of organic compounds was first introduced by James and Martin (*5, 6*) and has been actively explored by this laboratory (*3, 4, 12*) and by other workers (*1, 2, 7, 8, 11, 13*). Briefly, this method involves fractionation of materials with a column consisting of a nonvolatile solvent supported on a finely divided inert solid. Movement of the sample through the column is promoted and controlled by the flow of an inert carrier gas through the column. A small amount of the mixture to be analyzed is introduced at the head of the column, where it is absorbed into the immobile solvent and, as the flow of inert gas continues, the rate at which a given component of the mixture moves through the column is determined by its partial pressure over the immobile solvent and the rate of flow of the carrier gas. If the components of the mixture exert partial pressures that are significantly different, these components will move through the column at different rates, thereby causing separation to occur. Suitable detection and/or collection devices can be used at the end of the column either to identify or to collect the pure components as they emerge.

Although the technique has been demonstrated to be a new and powerful analytical tool in many applications, it seemed by nature to be particularly well suited to gas analysis. At the present time, a number of techniques are used to analyze hydrocarbon gases; among these are spectrometric (mass, infrared, ultraviolet), low temperature distillation, and the selective absorption methods. Spectrometric methods have clearly demonstrated their wide applicability to many types of gas analysis in recent years. However, the limitations of trained manpower and high initial cost place severe restrictions on the service these instruments can provide. The equipment required for low temperature distillation is somewhat less elaborate, but the analysis is very time-consuming and cannot, by itself, satisfactorily resolve complex mixtures of C_4 and C_5 hydrocarbons. Selective absorption techniques generally require relatively simple apparatus, but are best suited

for fixed gases and hydrocarbon-type analysis. In order to clarify the position which gas-liquid partition chromatography should be assigned relative to these other techniques, an apparatus was assembled to permit study of this method for analysis of the hydrocarbon gases.

APPARATUS

A schematic diagram of the apparatus is shown in Figure 1. It consists of the following principal components: the chromatographic column, thermal conductivity cells and associated bridge circuits to monitor the effluent of the column, an electronic recorder to record continuously the thermal conductivity of the emergent gas, a thermostatically controlled air bath to maintain the column and cell at constant temperature, and a system of valves, meters, and gages to allow the precise measurement of sample and to control the flow of sample and sweep gas into and through the column.

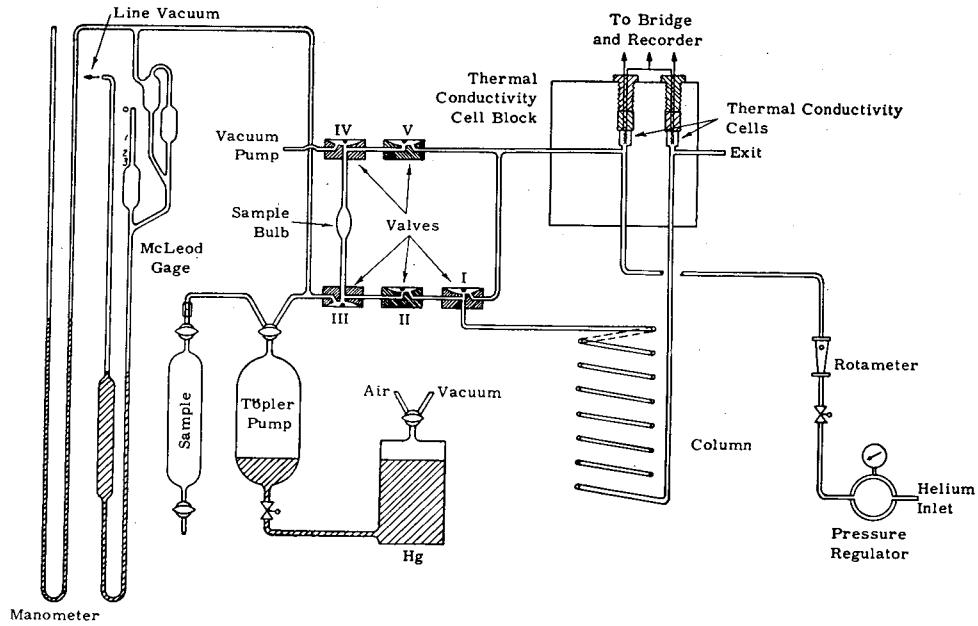

Figure 1. Schematic diagram of apparatus for gas analysis

The chromatographic column is made from a length of $^3/_8$-inch copper tubing that is coiled to fit conveniently into the air bath. The details of its packing, length, and operating conditions are described fully below. The constant-temperature air bath is constructed of Transite and metal and is well insulated with glass wool. Heat is provided by an electric heater that is connected to a variable transformer and cooling, when required, is supplied by mechanical refrigeration; a motor-driven fan supplies rapid air circulation. Bath temperature is controlled by a bimetallic expansion-type controller and the gas-liquid partition chromatography column, thermal conductivity cell block, sampling valves, and bulb are held at sufficiently constant temperature to maintain a steady signal to the recorder in the range of 0° to 50° C. The thermal conductivity cells are conventional Gow-Mac MT-T cells (Gow-Mac Instrument Co., 100 Kings Road, Madison, N. J.) mounted in a cell block of special design to minimize the effects of variations in flow; this cell block has been fully described (*3*). The direct current detection circuit is a conventional Wheatstone bridge. The preamplifier and recorder are manufactured by the Weston Electrical Instrument Corp. and are equipped with suitable variable shunt resistances to allow the full scale recording of 1, 2, 5, 10, 20, 50, or 100 mv. The sensitivity of the thermal conductivity cells is such that 0.1% concentrations of most hydrocarbon components can be readily determined in complex mixtures.

Referring to the right-hand side of Figure 1 helium is supplied to the system from a cylinder equipped with a two-stage reducing valve; a Moore pressure-regulating valve (Moore Products Co., Philadelphia, Pa.), surge tank (not shown), rotameter, and connecting lines deliver the gas to the reference thermal conductivity cell at a constant flow rate. From the reference cell the gas normally bypasses the sampling chamber and flows through valve I and into, through, and out the exit end of the column. The gas emerging from the column passes through the detecting thermal conductivity cell and is then vented to the atmosphere. The flow of helium is maintained in this manner at all times, except when a sample is being admitted to the column.

A gas sample is measured and admitted to the system in the following manner: The sampling system is evacuated by opening valves III and IV while valves II and V are in the closed position. During this operation the flow of helium through the column continues without interruption through valve I. The lines connecting the sampling chamber are evacuated to the stopcock of the sample tube through the three-way stopcock of the Töpler pump and the system is considered to be adequately evacuated when the pressure is reduced to 0.1 mm. or less, as indicated on the McLeod gage. The Töpler pump is communicated with the sampling tube through the two stopcocks and the sample is allowed to expand into the Töpler pump. Valve IV is turned to the off position to interrupt the action of the vacuum pump. The Töpler pump is then connected to the sampling chamber and manometer through the three-way stopcock of the Töpler pump. This causes sample to flow from the Töpler pump into the evacuated sampling system. The mercury level in the Töpler pump is adjusted to obtain the desired pressure in the sampling chamber, as indicated on the manometer. Valve III is closed next, shutting off the sample source and confining the sample to the sampling chamber and the connecting lines between valves II, III, IV, and V. In rapid succession, valve I is closed and valves II and V are opened, causing helium to flow through valves V, IV, III, II, and I, in the order listed. In this manner, the helium is caused to pass through the sampling chamber and its connecting lines, and the sample is swept into the column. Once the sample has been carried into the column, valve I may be opened and valves II and V closed; another sample can then be admitted to the sampling system while analysis of the preceding one is in progress.

Hoke stainless steel diaphragm valves are used throughout the system. Valves II and V are used without modification, but an additional port is drilled into the bodies of valves I, III, and IV to provide a low dead space, T (*4*). This sampling system is somewhat wasteful of sample; however, it is convenient and simple in operation. The sample size is readily adjusted by regulating its pressure; the Töpler pump, which serves both as a sample reservoir and a mixing device, holds sufficient sample for several replicate analyses. The sampling chamber has a volume of about 15 ml. and is generally filled at atmospheric pressure or less to give a sample size of 5 to 15 cc.-atm.

EXPERIMENTAL

Some attention has been given by others to the effects on the efficiency of gas-liquid partition chromotographic separations of operating variables such as temperature, pressure, carrier gas flow rate, size of column, type of inert support, kind and amount of immobile liquid, and size of sample (*1, 2, 5–8, 11, 12*). However, this field of analysis is in such a stage of development that it will probably be some time before the effects of the principal operating variables are completely evaluated. The conditions used in the present work were developed mainly by empirical means and have not been exhaustively studied, although they have made possible many successful separations.

Evaluation of gas analysis methods is always hampered by the difficulty with which complex gas mixtures of known composition are prepared in the laboratory. The present investigation was,

therefore, greatly aided by the availability of ten hydrocarbon mixtures of known composition which are marketed by the Phillips Petroleum Co. (Special Products Division, Bartlesville, Okla.). All ten mixtures were used in this work but, for brevity, four of the mixtures have been selected to illustrate the utility of this method.

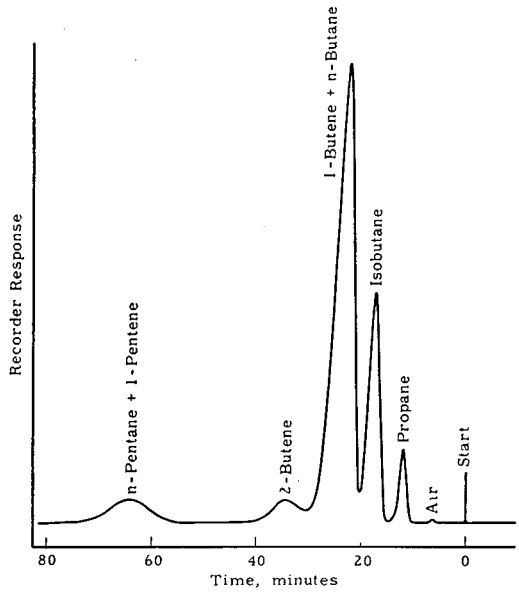

Figure 2. Chromatogram of Phillips mixture 35 using 6-foot diisodecyl phthalate column

The first column prepared to test the applicability of gas-liquid partition chromatography to the analysis of hydrocarbon gases was 6 feet in length and contained diisodecylphthalate (DIDP) as the stationary liquid. It was prepared in the following manner: Celite 545 (Johns-Manville) was acid-washed, dried, and mixed with diisodecylphthalate in an acetone slurry in a ratio of 40 grams of diisodecylphthalate to 100 grams of Celite. The acetone was removed on a steam bath and a sufficient quantity of the impregnated Celite was admitted to a 6-foot length of $^3/_8$-inch copper tubing to give a packing density of approximately 0.45 grams per ml. The copper tube was then coiled to fit conveniently into the air bath (ca. 8 inches in diameter) and connected to the apparatus as shown in Figure 1. Helium flow was adjusted to 45 ml. per minute and the air bath temperature was set at 35° C.; these conditions were arrived at somewhat arbitrarily after brief preliminary experiments indicated they would yield useful information.

A sample of Phillips mixture 35 was analyzed on the 6-foot diisodecylphthalate column and the resulting chromatogram is shown in Figure 2. It was observed that diisodecylphthalate served well for the separation of hydrocarbons in the order of their boiling points. However, because of the narrow boiling intervals between many olefins and paraffins having the same number of carbon atoms, the separations were not sharp for complex mixtures. Propane and propylene appeared as one peak, as did *n*-butane, isobutylene, and 1-butene. 2-Butene was separated from the other C_4 hydrocarbons, but no separation of the cis and trans isomers was apparent.

A search was made for a high-boiling stationary liquid which was more polar than the diisodecylphthalate, in the hope that the olefins would be retained more strongly in the liquid than the paraffins and thus effect more complete separation. Among the liquids tried, dimethylsulfolane (available in small quantities from Shell Chemical Corp., New York, N. Y.) (2,4-dimethyltetrahydrothiophene-1,1-dioxide) was selected on the basis of its performance. This compound retarded the olefins and gave excellent separation between olefins and paraffins of the same carbon number; however, the olefins were retarded to the extent that they overlapped the paraffins of the next higher carbon number.

These tests suggested the use of a two-stage column, with diisodecylphthalate serving as the stationary liquid to provide separation of hydrocarbons more or less by boiling point and dimethylsulfolane(DMS) serving to separate the olefins from the paraffins.

After several experiments, a two-stage column was prepared which consisted of the 6-foot diisodecylphthalate column in series with 16 feet of dimethylsulfolane on Celite 545 in the same ratio of 40 grams of solvent to 100 grams of supporting material. This column, when operated at 35° C. and a flow rate of 45 ml. of helium per minute, gave good separation of the paraffin hydrocarbons and retarded the olefins sufficiently to separate them from close-boiling paraffins; Figures 3 and 4 illustrate the separations obtained with Phillips mixtures 35 and 40. Inasmuch as the Phillips mixtures did not contain significant concentrations of the pentenes, a complex hydrocarbon mixture was prepared on a qualitative basis which contained most of the normally encountered hydrocarbons from C_1 to C_5, including five of the pentenes. In order to increase the efficiency of the separation

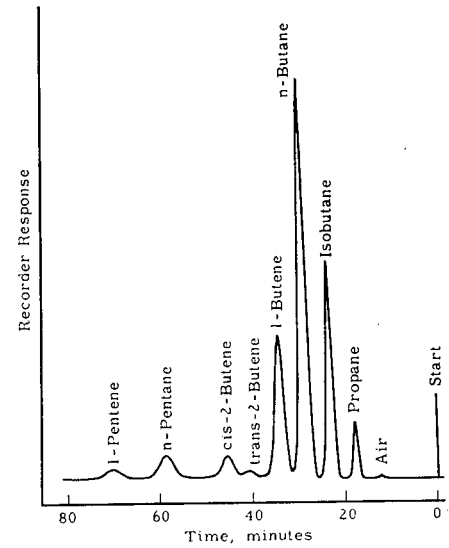

Figure 3. Chromatogram of Phillips mixture 35 using two-stage column at 35° C.

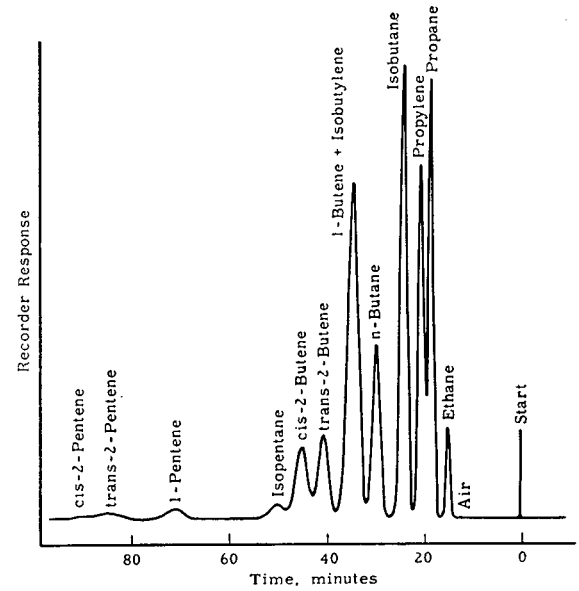

Figure 4. Chromatogram of Phillips mixture 40 using two-stage column at 35° C.

the bath temperature was lowered to 15° C., while the helium flow rate was maintained at 45 ml. per minute. The recorded chromatogram is shown in Figure 5. From this figure it is apparent that, under these operating conditions, good resolution of all the hydrocarbons through C_4 was obtained, with the exception of the 1-butene–isobutylene pair, and fair resolution of the pentenes was achieved. The total elapsed time for analysis of this mixture was 1 hour, 40 minutes. Two principal areas needing improvement were apparent from Figure 5 namely, resolution of the 1-butene–isobutylene pair and more complete separation of the pentenes.

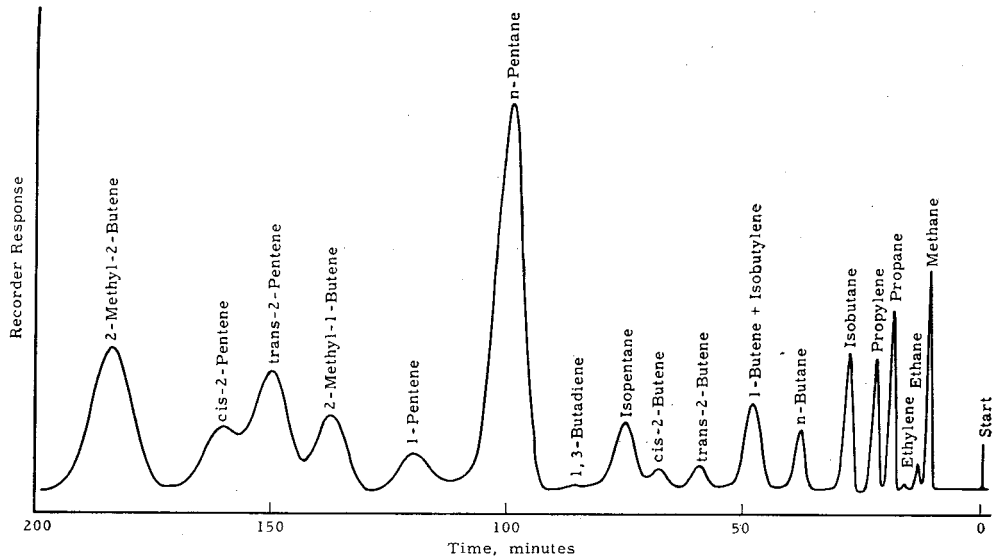

Figure 5. Chromatogram of complex hydrocarbon mixture using two-stage column at 15° C.

The pressure drop across a column using Celite 545 as the inert supporting material is in the order of 60 mm. per linear foot at a flow of 45 ml. per minute. In connection with the separation of close-boiling isomers, it appeared likely that rather long columns would be of interest. Others (*2*, *3*, *7*, *8*) faced with similar problems, sought a supporting material that was as effective as the Celite without its relatively high pressure drop. Their investigations revealed that Sterchamol, a German-made refractory brick, or Johns-Manville C-22 firebrick, pulverized and screened to a mesh size of 35 to 80, was an excellent substitute for Celite and exhibited less than half the pressure drop.

The separation of 1-butene from isobutylene was regarded with considerable interest in this work because of the importance of these olefins in the petroleum and petrochemical industries. Reviewing some of the preliminary work it was noted that dimethylsulfolane, in addition to being an excellent material for separating olefins from paraffins, also seemed to be a good fractionator for the olefins—e.g., the separation of *cis*- and *trans*-2-butene. Therefore, it was hypothesized that a long dimethylsulfolane column might be effective for separation of the close-boiling butylenes. Accordingly, a 25-foot column was packed with dimethylsulfolane, supported on C-22 firebrick in the same ratio of 40 grams of solvent to 100 grams of support, to test this hypothesis. The C-22 firebrick was found to pack quickly to a uniform density of 0.60 gram per ml. and was generally easier to handle than the Celite. A mixture of 1-butene and isobutylene was first tested with the column and the isomers were found to be about 60% separated. Encouraged by these results, a 50-foot column of the same material was prepared next

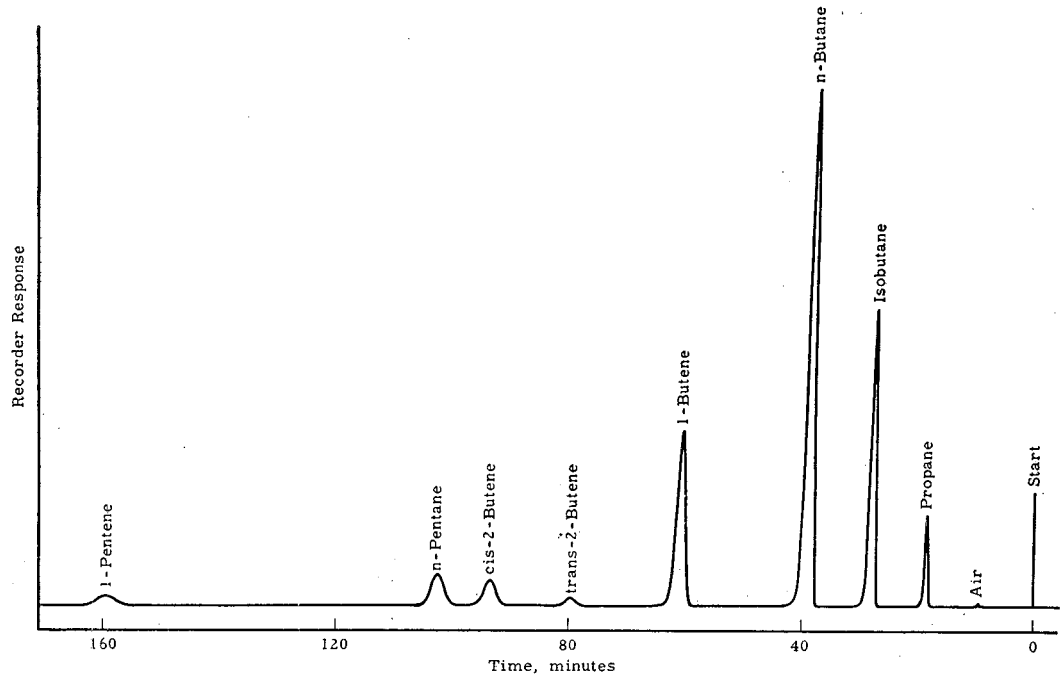

Figure 6. Chromatogram of Phillips mixture 35 using 50-foot dimethylsulfolane column at 0° C.

and separation of the pair was found to be essentially complete at 0° C. and a flow rate of 110 ml. of helium per minute.

To explore further the general usefulness of the 50-foot dimethylsulfolane column, Phillips mixtures 35, 37, 38, and 40 were tested; Figures 6 and 7 illustrate the type of separations that were obtained. It is evident that good resolution was obtained for most components. Incomplete separation was observed of propylene from isobutane, and of isopentane from *cis*-2-butene; however, these components are sufficiently separated to make an analysis still possible. Interestingly enough, 1-butene and isobutylene are separated on the dimethylsulfolane column in the reverse order of their boiling points. In view of the promising performance of this column, a 19-component mixture of light hydrocarbons was prepared and tested; the resulting chromatogram is shown in Figure 8. This test was also made at 0° C. with 110 ml. helium per minute; the total elapsed time was 3 hours. Referring back to Figure 5, which is a chromatogram of a similar mixture on the 22-foot combination diisodecylphthalate-dimethylsulfolane column, it is seen that the propylene-isobutane separation is less complete and that isopentane overlaps with *cis*-2-butene; however, isobutylene and 1-butene are completely separated by the long dimethylsulfolane column and considerable improvement is apparent in the pentene separation.

INTERPRETATION OF DATA

As other workers have discussed in detail (*5*, *6*), the emergence time of a given compound (or its retention volume) is constant under constant column conditions. The emergence time data which were accumulated during this study are summarized in Table I.

The possible use of peak height as a measure of component concentration has been discussed (*3*, *11*). The authors' experience confirmed the view that this parameter, without prior calibration, is generally unsuitable for quantitative analysis.

Considerably better results are obtained by using the areas under the peaks. The peaks in chromatograms obtained at

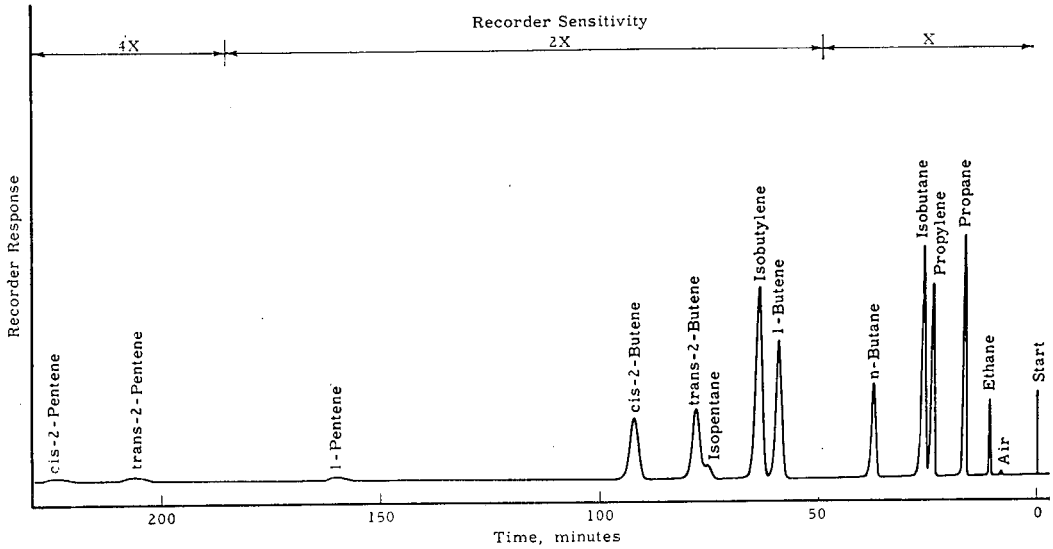

Figure 7. Chromatogram of Phillips mixture 40 using 50-foot dimethysulfolane column at 0° C.

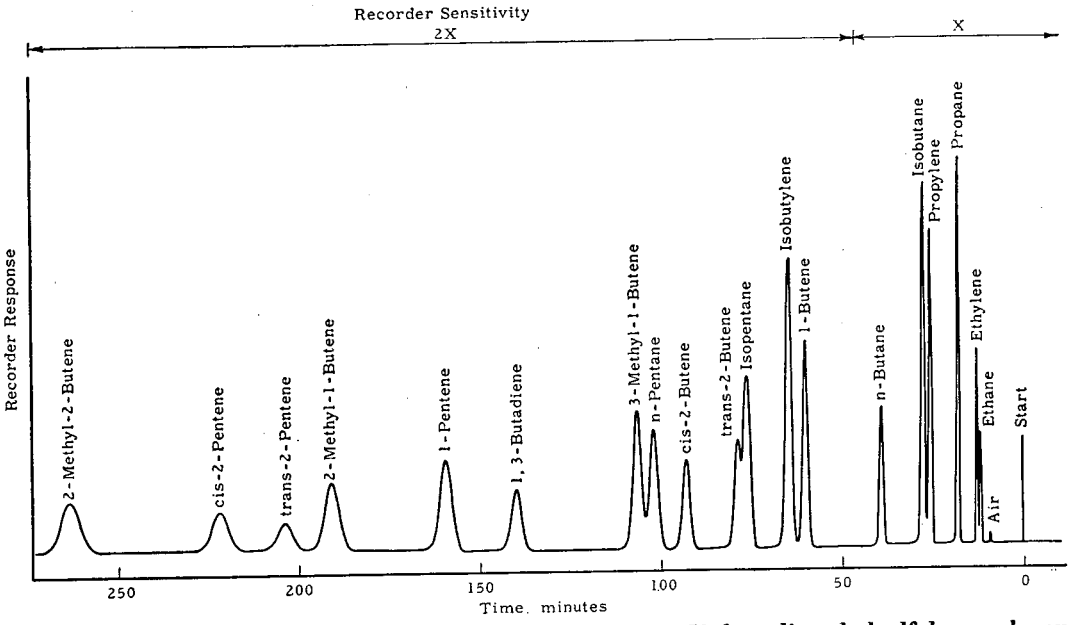

Figure 8. Chromatogram of 19-component mixture using 50-foot dimethylsulfolane column at 0° C.

suitable chart speed (0.5 inch per minute) were measured by means of a planimeter, and the ratios of individual peaks to total peak area were taken as a measure of the component concentrations. In the case of overlapping peaks, division of area was accomplished by drawing a vertical line from the lowest point between the two peaks to the base line. The resulting data are shown in Table II. It is evident that the distribution of area under the peaks correlates very well with weight per cent composition. Figures 2 to 8 show chromatograms obtained at low chart speeds which make the graphs more suitable for reproduction, but less suitable for quantitative evaluation.

DISCUSSION AND SUMMARY

The brief investigation described amply demonstrates that gas-liquid partition chromatography is a powerful new tool for the analysis of complex hydrocarbon gases. Separations are possible that are not conveniently made by any other technique. The emergence time of compounds under controlled operating conditions provides an excellent qualitative identification constant. Monitoring the thermal conductivity of the effluent gas indicates the emergence of hydrocarbon components with great sensitivity when helium is used as a sweep gas. The *thermal conductivity* recording also provides a basis for quantitative analysis of the mixture on an area distribution basis. The correlation of peak area with weight per cent is surprisingly good, considering the substantially different thermal conductivities of the hydrocarbons from ethane to pentane (*9*).

The measurement of peak area with a planimeter is a fairly tedious operation, particularly when applied to rather complex curves. These measurements should be greatly facilitated by use of an electromagnetic integrator, similar to that described by Lingane and Jones (*10*), for continuous measurement of the accumulated area under the curve. The use of peak height as a measure of component concentration, with adequate calibration for deviations from ideality, should not be ruled out. This technique can be very useful when the operating conditions are maintained constant; calibrations are applicable only to the operating conditions under which they are made. Peak height measurement should be a particularly useful technique in control analysis, where detection of changes in the concentration of critical components in a product mixture is of primary importance. Tests by this method yield precisely reproducible curves.

From the chromatograms shown in Figures 2 to 8 it is evident that very different information is obtained for the three column systems that have been tested. It is unlikely that any single type of column would satisfy the analytical requirements for all situations. Primary factors that must be considered are the type and complexity of the sample and the analytical information that is required. Clearly the dual 22-foot column that was tested would provide no useful information in the analysis of a mixture of isobutylene and 1-butene. Similarly, the 50-foot dimethylsulfolane column would be a poor choice where a clean separation of isopentane from *trans*-2-butene is desired. On the other hand, the 6-foot diisodecylphthalate column might serve very well for the analysis of materials that were free of olefins, and to use the 50-foot dimethylsulfolane column for this analysis would unnecessarily prolong the analysis.

Column stability is an important factor in gas-liquid partition chromatography, because deterioration of the column packing can adversely affect its performance. This can evidence itself in poorer fractionation and/or a change in emergence time of the components. The column packings which were used in this investigation showed excellent stability. The dual column, containing 6 feet of diisodecylphthalate and 16 feet of dimethyl-

Table I. Emergence Times under Various Operating Conditions

Compound	Emergence Time, Min.[a] 6-foot DIDP 35° C., 45 ml./min.	6-foot DIDP–16-foot DMS 35° C., 45 ml./min.	6-foot DIDP–16-foot DMS, 15° C., 45 ml./min.	50-foot DMS, 0° C., 110 ml./min.
Methane	7	12	12	9
Ethane	8	14	14	12
Ethylene	8	15	16	13
Acetylene	..	22	27	51
Propane	12	19	19	18
Propylene	12	20	22	26
Isobutane	17	23	28	28
n-Butane	22	29	38	40
1-Butene	22	34	48	61
Isobutylene	22	34	48	66
trans-2-Butene	34	40	59	80
cis-2-Butene	34	45	68	94
1,3-Butadiene	..	54	86	141
Isopentane	49	48	75	78
n-Pentane	62	60	100	103
1-Pentene	62	72	119	160
3-Methyl-1-butene	..	..	..	108
2-Methyl-1-butene	..	79	137	192
trans-2-Pentene	..	85	149	205
cis-2-Pentene	..	90	160	223
2-Methyl-2-butene	..	102	182	268
2-Methyl-1,3-butadiene	..	..	..	390

[a] Time measured from injection of sample to apex of component peak.

Table II. Comparison of Area Distribution with Known Weight Per Cent Values

Hydrocarbon	Phillips Mixture 35 Phillips values	Phillips Mixture 35 Analysis on DIDP-DMS column[a]	Phillips Mixture 35 Analysis on DMS column[b]	Phillips Mixture 37 Phillips values	Phillips Mixture 37 Analysis on DIDP-DMS column[a]	Phillips Mixture 37 Analysis on DMS column[b]	Phillips Mixture 38 Phillips values	Phillips Mixture 38 Analysis on DIDP-DMS column[a]	Phillips Mixture 38 Analysis on DMS column[b]	Phillips Mixture 40 Phillips values	Phillips Mixture 40 Analysis on DIDP-DMS column[a]	Phillips Mixture 40 Analysis on DMS column[b]	19-Component Mixture Blending data	19-Component Mixture Analysis on DMS column[b]
Ethane	..	..	..	..	..	..	..	..	..	2.4	2.4	2.2	1.3	1.5
Ethylene	..	..	..	..	..	..	..	..	..	..	..	..	2.0	2.7
Propane	3.9	4.2	4.5	..	..	..	8.6	9.6	9.4	13.7	13.0	13.4	7.4	8.3
Propylene	..	..	..	..	..	..	..	..	..	13.8	14.2	14.6	7.4	8.1
Isobutane	20.1	19.2	20.3	3.1	2.9	2.8	14.5	15.0	15.4	21.2	20.3	20.1	11.5	11.8
n-Butane	44.2	43.6	44.5	14.6	14.7	14.9	29.3	29.4	30.6	9.2	9.6	9.9	5.0	5.3
1-Butene	19.5	20.4	19.5	16.9	..	16.9	..	..	..	9.6	..	10.0	5.3	5.5
Isobutylene	..	..	..	7.0	..	7.0	..	..	..	15.3	..	15.3	8.3	8.4
1-Butene + isobutylene	..	..	..	(23.9)	23.4	(23.9)	..	..	..	(24.9)	25.0	(25.3)	..	..
trans-2-Butene	..	1.3	1.5	22.2	22.1	22.0	..	..	..	..	6.2	6.0	..	3.1
cis-2-Butene	..	3.9	3.8	19.0	19.7	19.6	..	..	..	..	6.5	6.4	..	3.6
Total 2-butenes	4.9	(5.2)	(5.3)	..	..	..	..	..	..	11.6	(12.7)	(12.4)	6.3	(6.7)
1,3-Butadiene	..	..	..	17.3	17.2	16.6	..	..	..	..	..	..	3.4	3.4
Isopentane	..	..	..	..	..	..	15.7	14.9	14.7	1.4	1.6	0.8	6.5	6.0
n-Pentane	5.1	5.1	4.4	..	..	..	31.7	31.1	30.0	..	..	..	5.7	5.1
1-Pentene	2.4	2.3	1.7	..	..	..	..	..	..	0.7	0.7	0.6	5.8	5.4
3-Methyl-1-butene	..	..	..	..	..	..	..	..	..	..	..	..	6.7	6.2
2-Methyl-1-butene	..	..	..	..	..	..	..	..	..	..	..	..	5.7	5.2
trans-2-Pentene	..	..	..	..	..	..	..	..	..	..	0.4	0.4	..	1.9
cis-2-Pentene	..	..	..	..	..	..	..	..	..	..	0.2	0.3	..	3.3
Total 2-pentenes	..	..	..	..	..	..	..	..	..	0.7	(0.6)	(0.7)	5.9	(5.2)
2-Methyl-2-butene	..	..	..	..	..	..	..	..	..	..	..	..	5.7	5.2

[a] 35° C.
[b] 0° C.

sulfolane packing, was used continuously for several months at 35° C. and showed no change in performance.

The problem of determining nonhydrocarbon gases has received little consideration in this investigation. At present it is difficult to visualize a column that will separate the uncondensable gases from one another as well as the individual hydrocarbons. A simple cold-trap separation of such samples might provide a hydrocarbon fraction for analysis by gas-liquid partition chromatography and an uncondensable fraction for adsorption chromatography (*11*). Another possible approach is separation of the uncondensable gases in a single fraction with methane by gas-liquid partition chromatography, and analysis of this fraction by mass spectrometry. The design of suitable equipment for sampling the effluent gas from the thermal conductivity cell will greatly aid this approach. Such equipment will also be valuable for collecting hydrocarbon components when further qualitative identification is required.

ACKNOWLEDGMENT

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GAS CHROMATOGRAPHY

Use of Liquid-Modified Solid Adsorbent to Resolve C_5 and C_6 Saturates

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A new type of column packing for gas chromatography consists of an active solid to which a small amount of liquid solvent has been added. The amount of solvent is sufficient to prevent or greatly reduce the tailing of chromatographic peaks normally encountered with active solid supports but not to replace the solid as the principal sorbing medium. Such liquid-modified solid adsorbents therefore resemble unmodified solids in retaining paraffins relative to naphthenes. Thus, the C_6 naphthenes emerge ahead of the C_7 paraffins, in contrast to the behavior in gas-liquid partition columns, where the reverse sequence is observed. Usefulness of the new type of column is exemplified by the analysis of a mixture containing all ten of the commonly encountered C_5 and C_6 saturates. A 50-foot column of a commercial carbon black (Pelletex) containing 1.5% squalane (hydrogenated squalene) resolved all components, including 2,3-dimethylbutane and 2-methylpentane, which could not be separated with any of a large number of partition-type columns tested. The time for analysis is about 2 hours and the average error has been about 0.5%.

QUANTITATIVE resolution of saturated hydrocarbons into types and individual constituents has long been a problem of practical importance. For type estimations several methods have been more or less thoroughly explored, including empirical formula, refractive index–density relations, liquid-liquid adsorption chromatography, thermal diffusion, and solvent partition. However, these methods have been only moderately successful and do not offer much promise of yielding a more detailed analysis. Infrared and mass spectrometry give essentially all individual compounds, possibly up to C_9, with excellent results, but extensive sample preparation by chromatography and fractional distillation is required at least for C_7 and higher hydrocarbons.

A new approach to the problem of analysis of hydrocarbons has become available with the rapid development of gas chromatography. By means of this fascinating tool encouraging results have been obtained for C_5 and lower hydrocarbons both by gas-liquid partition (*4*, *9*, *11*) and by gas-solid adsorption chromatography using solid adsorbents (*5*, *10*, *11*). In gas-liquid partition chromatography the sample components are separated by their differing solubilities in the liquid film on the support, and adsorption effects of the support are generally negligible. In the adsorption scheme, on the other hand, no liquid is used, and the separation is based on differing adsorbabilities of the components on the solid surface.

With increasing molecular weight the resolution of individual compounds is of course complicated by the rapid rise in the number of isomers. Nevertheless, reported results are encouraging. For example, Keulemans and Kwantes (*8*) have demonstrated that normal and isoparaffins through C_9 can be distinguished by gas-liquid partition chromatography, provided the naphthene content is low. James (*7*) also employed the technique to determine C_5 and C_6 constituents in commercial petroleum ethers and was able to separate many of the individual components. Thus, it was shown that gas chromatography has great potentialities for hydrocarbon analysis, and it seemed desirable to make a thorough study of the behavior of various column types as applied to hydrocarbons. In this study a suitable column and technique were developed for the separation of C_5 and C_6 saturates.

Gas-liquid partition chromatography columns were first investigated, generally with Celite-type supports and 40% added liquid. Although a wide variety of liquids was tested, many of which gave excellent resolution in general, none was found which would separate 2,3-dimethylbutane (boiling point 58.0° C.) and 2-methylpentane (60.3° C). Furthermore, all the partition columns retarded naphthenes relative to paraffins, so that cyclohexane, and often methylcyclopentane, emerged after the first C_7 paraffins. On the other hand, all solid adsorbents were found

to retard paraffins relative to naphthenes, as might be expected from the liquid-solid adsorption work of Hirschler and Amon (*6*). These authors showed, for example, that both silica gel and carbon adsorb *n*-heptane more strongly than methylcyclohexane from 1 to 1 mixture, and that silica gel adsorbs 2,4-dimethylpentane preferentially to cyclohexane. In gas chromatography using solid adsorbents it was observed that all of the C_6's emerged well ahead of the C_7's, and some adsorbents resolved the troublesome pair, 2,3-dimethylbutane and 2-methylpentane.

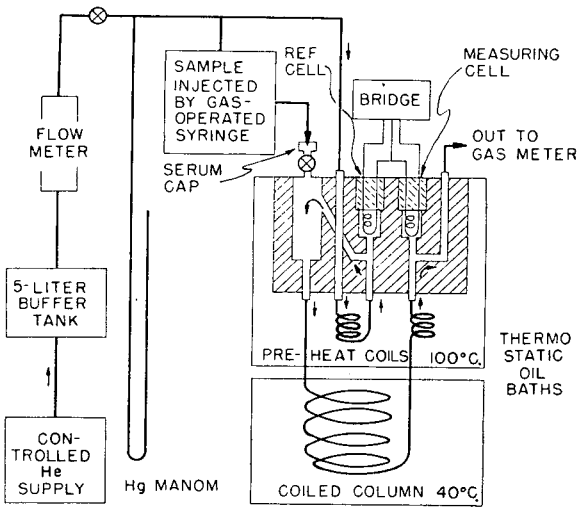

Figure 1. Schematic diagram of gas chromatographic apparatus

Complete separations were difficult to obtain with solid adsorbents owing to overlapping of the peaks, caused by tailing. However, it was found that tailing could be reduced or virtually eliminated by adding a small amount of a high-boiling or strongly adsorbed liquid to the adsorbent. With suitably low amounts of liquid, paraffins are retarded relative to naphthenes, as with the dry adsorbents. Therefore these new packings may be viewed as liquid-modified solid adsorbents. Of course, as more liquid is added the packing approaches the gas-liquid partition chromatography type, until finally the effect of the surface of the active support is generally nullified.

Of the modified adsorbent packings tested for C_5 and C_6 saturates, which include small amounts of various liquids on carbons, silica gel, and Celites, the best was 1.5% squalane (a branched paraffin, $C_{30}H_{62}$) on Pelletex (a furnace black). The results obtained with this packing in the analysis of C_5-C_6 mixtures are discussed below. The performance of this and other columns will be compared in more detail in a subsequent publication.

EXPERIMENTAL

Apparatus. The apparatus used was conventional, with certain modifications described by Dimbat, Porter, and Stross (*3*). A flow diagram is given in Figure 1.

The sensing elements were thermal conductivity cells (Gow-Mac Instrument Co., Madison, N. J.) of the convection-diffusion type (nonflow-sensitive). The potentiometer was operated at 5 or 10 mv. for full scale deflection. The column and the conductivity cell block were contained in separate thermostats (silicone oil) at 40° and 100° C., respectively, and the two units were connected by short pieces of 1/8-inch copper tubing. Samples were injected directly into the cell block by means of a syringe connected to the carrier gas inlet line. Helium was used as carrier gas at a flow rate of about 20 ml. per minute, and when it was desired to compare emergence times, the measured values were recalculated to a flow rate of exactly 20 ml. per minute at 20° C. The inlet pressure was 280 mm. for the 50-foot column employed and proportionally less for the 10-foot columns tested.

Pelletex. This material, which was used as the adsorbent, is a pelleted furnace black (Godfrey Cabot Co., Boston, Mass.) of surface area 24 sq. meters per gram, screened to 14–48 mesh.

Squalane. This $C_{30}H_{62}$ paraffin (2,6,10,15,19,23-hexamethyltetracosane) of molecular weight 423 and boiling point 210° C. at 1 mm., is obtained by hydrogenation of squalene (an acyclic isoprenoid ex-shark liver oil) over a platinum catalyst at 200 to 1000 pounds per square inch. Its high boiling point, good thermal stability, and low viscosity make it an especially attractive liquid of the paraffin type.

Preparation of Columns. The packing was made from 157 grams of Pelletex (350 ml.), 14–48 mesh, and 2.36 grams of squalane in about 275 ml. of petroleum ether. The squalane solution was added to the Pelletex so as to wet all particles, and the petroleum ether was evaporated on a steam bath, followed by drying at 110° C. for 1 to 2 hours. The dry material was packed with the aid of an electric vibrator into both ends of a 50-foot by 1/4-inch (outside diameter) copper tubing, folded into U-shape. The ends were then plugged with glass wool and the tubing was wound into a coil of four concentric helices on a 1 3/8-inch tube as a mandrel, the finished coil being about 7 inches long and 3 1/2 inches wide. Two 10-foot columns were also prepared, one with and one without squalane.

Hydrocarbon Test Samples. Phillips Petroleum Co. "pure" hydrocarbons (99% minimum) were used.

RESULTS AND DISCUSSION

The effectiveness of 1.5% squalane for reducing tailing on Pelletex is illustrated by Figure 2, where chromatograms for a 2 to 1 mixture of 2,4-dimethylpentane and cyclohexane on dry and modified Pelletex are presented. The greater peak symmetry obtained with the modified Pelletex column shows that the adsorption isotherm is more nearly linear than with the bare solid. Squalane affected the separation in two additional ways: The adsorptivity of the solid was reduced, as evidenced by the lower temperature required for comparable emergence times, and the paraffin emerged sooner relative to the naphthene.

To illustrate the behavior of the squalane on Pelletex column, the results of the analysis of three synthetic blends of C_5-C_6

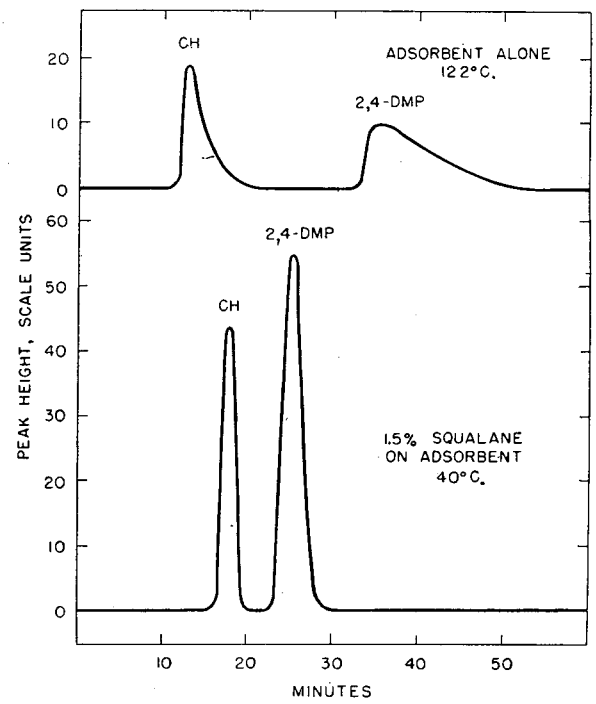

Figure 2. Effect of tailing reducer in gas chromatography

10-foot × 1/4-inch Pelletex
2,4-DMP + CH, 2 to 1
1.5-mg. sample
Flow rate, 20 ml. per minute
20 scale units = 1 mv.

Table I. Gas Chromatographic Analyses of C_5-C_6 Saturates Blends

Column, 50 feet × 1/4 inch, 1.5% squalane on Pelletex
Temp., 40° C.
Pressure, 280 mm.
He flow, 20 ml. per minute
Sample, 10 mg.

Sample	Component, Wt. % 2-MB	*n*-Pentane	CP	2,2-DMB	2,3-DMB	2-MP	3-MP	MCP	*n*-Hexane	CH	Av. Error, %
No. 1											
Present	..	..	..	4.9	10.2	15.6	21.3	5.9	42.1	..	..
Found[a]											
Planim.	..	..	..	4.6	9.5	14.8	22.3	6.6	42.0	..	0.6
HW[b]	..	..	..	4.7	10.0	15.3	22.1	6.5	41.4	..	0.5
No. 2											
Present	..	..	..	2.5	1.2	3.6	2.4	..	90.3	..	..
Found[c]											
Planim.	..	..	..	2.3	1.2	3.3	3.0	..	90.2	..	0.2
HW[b]	..	..	..	2.3	1.3	3.4	3.1	..	90.0	..	0.3
No. 3											
Present	2.4	5.7	8.9	3.4	7.1	10.8	14.7	4.1	29.0	13.9	..
Found[c]											
Planim.	2.8	5.7	9.6	3.3	6.8	10.0	14.8	4.4	28.6	14.0	0.3
HW[b]	2.7	5.7	9.4	3.3	6.8	10.5	15.1	4.4	28.5	13.6	0.3

[a] Average of triplicates.
[b] Product of peak height and peak width at half height.
[c] Average of duplicates.

saturates are shown in Table I. An adsorptogram representing the resolution of a sample containing all the commonly encountered C_5 and C_6 saturates is reproduced in Figure 3. (Numerous cyclopropanes and cyclobutanes lie in this boiling range. Their occurrence is rare, however, at least in petroleum, and they have therefore been neglected here.) It is seen that virtually complete separation of all ten components was achieved and that the last C_6 emerged well ahead of the first C_7, the latter (when present) being indicated by the dotted curve. The elapsed time for the analysis was about 2 hours. The sharpness of peaks in these experiments was comparable with that obtained with the best gas-liquid partition chromatography columns.

The peak separations became poorer with increasing sample size and temperature. Therefore not more than about 20 mg. of sample or 3 mg. of close-neighboring components should be charged, and a temperature of 40° C. or lower is preferred. Column length is another important factor, for in order to achieve these separations it was necessary to extend the length from an initial of 10 feet to 50. Longer columns were not tried, but might be expected to give still better separations. The effect of carrier gas flow rate was not investigated; the rate of 20 ml. per minute was selected, because it had been found suitable in studies with other types of 1/4-inch columns.

The percentage of each component was determined by measuring the areas under the peaks and calculating the per cent of total for each, assuming the areas to be a measure of the weights of the hydrocarbons present. Measurements were made both by means of a planimeter (extrapolating the beginning or end of the peaks where necessary) and by multiplying peak height by width at half height (HW) (*1*). The results were about equally accurate, the average error being 0.3 to 0.5% in both cases, and the maximum 1% (Table I). The weight by width method is preferred because it is faster.

As expected, the paraffins emerged in the order of their boiling points, but the three naphthenes emerged with lower boiling paraffins. Thus, cyclopentane (boiling point 49° C.) emerged well ahead of 2,2-dimethylbutane (50°), cyclohexane appeared before 2,4-dimethylpentane (both 81°), and methylcyclopentane (72°) before *n*-hexane (69°). Therefore, with respect to behavior

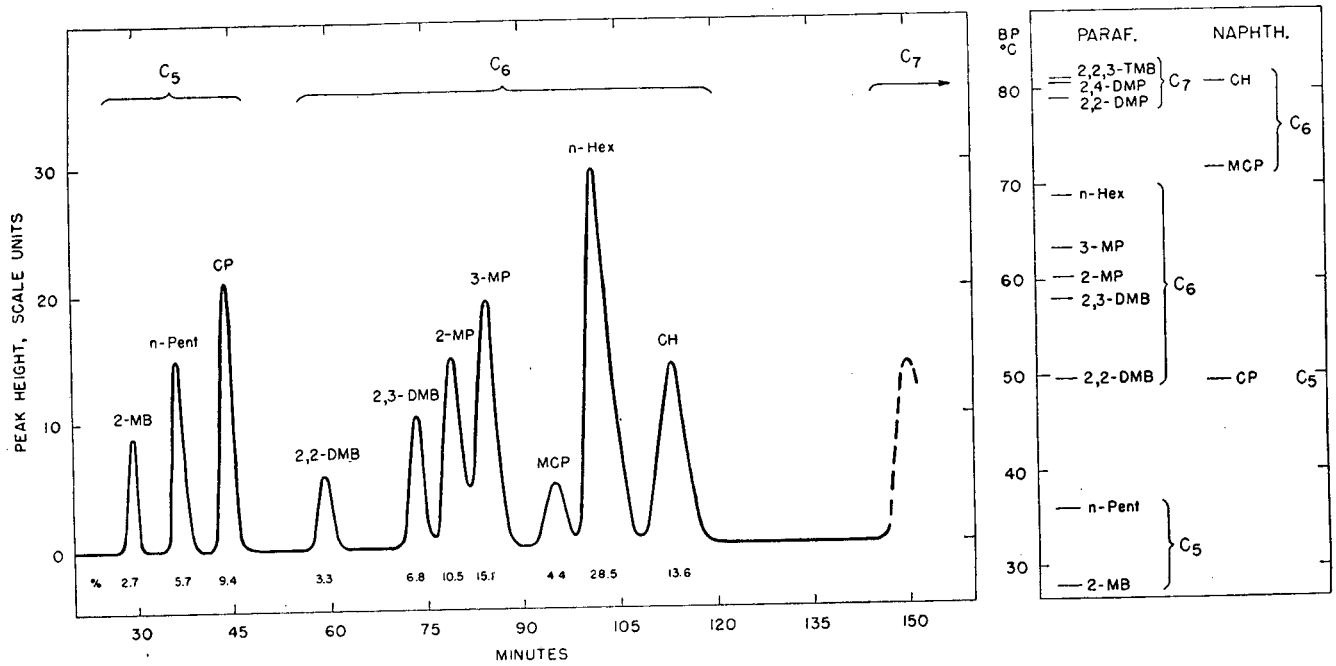

Figure 3. Gas chromatographic analysis of C_5 and C_6 saturates

Sample 3
1.5% squalane on 50-foot × 1/4-inch Pelletex
40° C.
280-mm. pressure
10-mg. sample
Flow rate, 20 ml. per minute
20 scale units = 1 mv.

toward naphthenes, the 1.5% squalane on Pelletex column is more nearly like a solid adsorbent than like a liquid-type column which retards naphthenes.

Although the liquid-modified Pelletex column has proved useful for analysis of C_5 and C_6 saturates, probably the chief value of this type of column is that it separates naphthenes from paraffins in a manner opposite to gas-liquid partition chromatography columns, and therefore offers new possibilities for analysis of saturates. Thus, a "carbon number" analysis is possible, at least through C_7, according to the authors' experience, and probably higher. When a further breakdown or type analysis is desired, the carbon number cuts can be separated into naphthenes and paraffins by means of a highly polar gas-liquid partition chromatography column, such as glycol, which greatly retards naphthenes.

A few olefins and benzene were tested as to emergence time through the squalane on Pelletex column. These results are listed below, together with those for saturates of similar boiling point.

Hydrocarbon	Boiling Point, ° C.	Emergence Time, Min. at 20 Ml./Min.
1-Pentene	30	38
n-Pentane	36	36
1-Hexene	64	91
n-Hexane	67	102
Benzene	80	114
Cyclohexane	81	113
Cyclohexene	83	132

With the liquid-modified Pelletex column unsaturation has little effect on the emergence time. All the C_6 ring compounds emerge ahead of the C_7 band, which begins at about 150 minutes, as shown in Figure 3. Therefore this column is suitable for carbon number analysis of a mixture containing all three types of hydrocarbons.

Interfering olefins and aromatics can be removed and a representative sample of saturates obtained for gas chromatographic analysis by liquid phase fluorescent indicator chromatography using a column similar to that described by Criddle and Le Tourneau (*2*). It consists of four sections of inside dimensions (top to bottom) 150 × 25, 350 × 10, 350 × 5, and 700 × 2 mm., the three lower sections containing Davison's grade 923 silica gel. Using a syringe to reduce evaporation losses, 2 to 3 ml. of sample are introduced below the gel surface and eluted with isopropyl alcohol under a pressure of 1 to 2 pounds per square inch. The saturates are collected in a chilled vial, protected from air, and the cut is taken when the first drop of yellow-green fluorescent dye forms at the tip of the column as observed in ultraviolet light in a dark room. This technique was tested with a blend of C_5-C_7 saturates with olefins from a catalytically cracked gasoline; gas chromatographic analysis showed a maximum change of 1% in the composition of the recovered materials.

For the analysis of C_5 and C_6 saturates the gas chromatographic method described has certain advantages over spectrometric methods. Because saturates outside the C_5-C_6 range do not interfere, as they do in spectrometry, extreme care in fractionation is not required. Furthermore, the presence of such higher or lower boiling constituents can be detected. However, in the absence of C_7's the infrared method is roughly comparable with the gas chromatographic method in accuracy and time per analysis. Another important advantage of the gas chromatographic method is that the apparatus is relatively simple and does not require a highly skilled operator.

It is concluded that liquid-modified adsorbents, such as the squalane-Pelletex column employed here, constitute a useful addition to the media available for gas chromatographic separations. Columns of this type differ markedly from the more familiar gas-liquid type columns in respect to the sequence of resolution of saturates. They are also "flexible," for by varying the amount of liquid the naphthene peaks can be moved relative to the paraffin peaks almost at will. The resolution is excellent and comparable with that of the best gas-liquid type columns tested.

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Mass Spectrometric Analysis

Broad Applicability to Chemical Research

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The heated-inlet mass spectrometer, applied in the past mainly to petroleum compounds, is an analytical tool of broad applicability to the whole field of chemistry. Quantitative analysis approaching the high accuracy and number of components found previously with light hydrocarbons is possible. Positive identification of unknown components of complex mixtures is illustrated. Unique information on molecular structure is provided, and complete structure determination is often possible without standards. It is hoped to show that the mass spectrometer provides a valuable complement to the rapidly growing field of instruments capable of analyzing a wide diversity of samples.

THOUGH the primary use of the analytical mass spectrometer has been in routine quantitative analysis of light hydrocarbons, a number of investigators have reported on the unique qualitative and quantitative applications to the general field of chemistry, especially organic chemistry. The analytical advantages of the mass spectrometer for volatile oxygenated compounds (*14, 18, 21, 22, 35, 36*), thiophenes (*19*), aromatic hydrocarbons (*19*), lactones (*13*), acids (*16*), haloalkanes (*1, 26, 35*), amines (*7*), and metallo-organic compounds (*8, 9*) have been described. Significance of the spectra of such compounds as ketene dimer (*23*), pentaborane (*10*), diborane (*28*), dimethylphosphinoborine trimer (*12*), phenol, thiophenol, and aniline (*27*) has also been discussed.

Table I. Mass Spectra of Polyisopropylbenzenes

m/e	Tetra-isopropyl-benzene	Tri-isopropyl-benzene	Diisopropyl-benzene	Isopropyl-benzene
105	11	31.4	61	368
119	8	25.4	105	3.31
120	1	2.6	10.6	100
132	1	1.7	13.2	
133	6	18.3	4.8	
146	3	2.3	2.6	
147	14	23.3	344	
160	1	1.8	0.47	
161	4	109	2.4	
162	0.6	14.6	100	
174	0.2	1.8		
175	0.7	6.7		
188	..	2.7		
189	..	338		
202	2.7	0.19		
203	24	3.0		
204	4.0	100		
216	1.3			
217	0.69			
230	36			
231	301			
244	6.9			
245	5.7			
246	100			
S_P/S_{Tol}	0.23	0.26	0.28	0.39

The mass spectrum indicates the amount and mass of the positive ions formed by electron bombardment of a sample. This unique information concerning the concentration and kind of molecules in a sample has been of only limited use in these general fields of chemistry because relatively few compounds of these types give sufficient vapor pressure (at least 0.05 mm.) to be introduced as a gas into the ionization chamber of the ordinary mass spectrometer. The development of heated-inlet systems (*3, 6, 29, 36*) has made possible the analysis of compounds of much lower volatility by heating the sample at 200° to 400° C. to obtain the necessary vapor pressure. The few instruments of this type described have been used mainly for hydrocarbons of high molecular weight, though applications to ketones (*32*) and alcohols (*4*) have been demonstrated.

This paper points out the value and uniqueness of the quantitative and structural information obtainable with the mass spectrometer on the broad range of compounds that can now be analyzed with the heated-inlet mass spectrometer. This has apparently been little appreciated up to the present because of the unavailability of heated instruments and the high interest in application of most of these to petroleum hydrocarbons.

EXPERIMENTAL

The spectra given were obtained on two 90° sector-type mass spectrometers (*6*). Their inlet systems are heated at 100° and 200° C., respectively, and solid samples placed in Teflon capsules can be directly introduced into the 200° system through a vacuum seal (*5*). No effort was made to run all the spectra given in this work on the same instrument or under exactly the same operating conditions, as they can be only of semiquantitative use for comparison with other instruments. However, comparison with spectra from a Consolidated Engineering Corp. Model 21-103 mass spectrometer in this laboratory shows striking similarity for many compounds such as aromatic hydrocarbons. Peaks below 1% of the height of the highest have been omitted in the graphically presented spectra of pure compounds because of their doubtful structural significance. However, the instruments used are in general capable of recording peaks less than 0.01% of the highest. Evacuation of the sample-handling system for 10 to 20 minutes is usually sufficient to lower peaks from the previous sample below this level.

QUANTITATIVE ANALYSIS

The high accuracy obtainable in the mass spectrometer analysis of mixtures containing as many as 30 components has been utilized for years for light hydrocarbon and inert gases. A natural extension of this with the heated instruments in this laboratory has given very useful quantitative analyses of mixtures not easily resolved by other methods. The analysis of the reactor products from the alkylation of benzene with propylene is an example of this. A typical "bottoms" sample might contain 15% mono-, 70% di-, 15% tri-, and 5% tetraisopropylbenzene and higher. Table I shows the relative heights of the important peaks in the mass spectra of the principal constituents. The parent peaks (molecular ions) have been designated as 100 for convenience in calculation. "S_P/S_{Tol}" refers to the relative sensitivity of the compound, in scale divisions of the parent peak per milligram of sample, as compared to the sensitivity of the *m/e* 92 peak of toluene. Minor components, such as substituted styrenes and indanes, are also detected and reported. Calculation for this simple type of analysis is carried out by the usual stepwise subtraction of component spectra (*37*).

The mass spectra of the products of the ethylation of dichlorobenzene are given in Table II. Halogen isotope peaks here and in Table IV have been omitted for brevity. Analyses of synthetic blends of these components, shown in Table III, illustrate that accuracies are obtainable approaching those found with light hydrocarbon analysis by the mass spectrometer. The crude alkylate was calculated on the weight of sample introduced, so that the amount of material with insufficient vapor pressure to register on the spectrum (tars) could be estimated by difference.

An example of the spectra used in analyzing perhalogen compounds is shown in Table IV. The sensitivities are calculated for the highest, or base, peaks. These compounds are products of

Table II. Mass Spectra of Ethylated *p*-Dichlorobenzenes

m/e	Tetraethyl-dichloro-benzene	Triethyl-dichloro-benzene	Diethyl-dichloro-benzene	Ethyl-dichloro-benzene	Dichloro-benzene
139	4.31	7.53	12.8	128	..
145	1.34	1.71	0.04	0.41	0.40
146	0.32	0.20	0.33	2.32	100
159	1.53	3.46	9.0	208	
167	2.46	3.18	72.2	..	
173	2.65	5.98	10.6	2.16	
174	0.35	0.97	3.67	100	
187	6.23	12.7	164		
195	0.80	41.0	.		
201	2.52	13.6	1.39		
202	0.22	1.53	00		
215	3.67	151			
223	20.8	..			
229	8.69	1.44			
230	1.21	100			
243	127				
258	100				
S_P/S_{Tol}	0.25	0.24	0.22	0.20	0.75

Table III. Analysis of Known Mixtures of Ethylated Dichlorobenzene

	Flashed Alkylate		Crude Alkylate	
	Synthesis	By M.S.	Synthesis	By M.S.
Dichlorobenzene	63.2	63.2	58.8	59.3
Ethyldichlorobenzene	30.8	30.9	28.7	28.4
Diethyldichlorobenzene	5.9	5.8	5.5	5.3
Triethyldichlorobenzene	0.08	0.10	..	..
Tetraethyldichlorobenzene	...	0.01	..	..
Nonvolatiles (tars)	...	...	7.0	7

Table IV. Mass Spectra of Bromochloromethanes

m/e	Ion	CBr_4	CBr_3Cl	CBr_2Cl_2	$CBrCl_3$	CCl_4
117	CCl_3—	..	..	..	100	100
152	CCl_4	..	..	..	..	0.00
163	$CBrCl_2$—	..	0.56	100	67.2	
198	$CBrCl_3$	..	..	..	<0.03	
207	CBr_2Cl—	..	100	18.0		
242	CBr_2Cl_2	..	..	0.02		
251	CBr_3—	100	5.5			
288	CBr_3Cl	..	0.09			
332	CBr_4	0.10				
	S_B/S_{Tol}	0.25	0.20	0.30	0.10	0.30

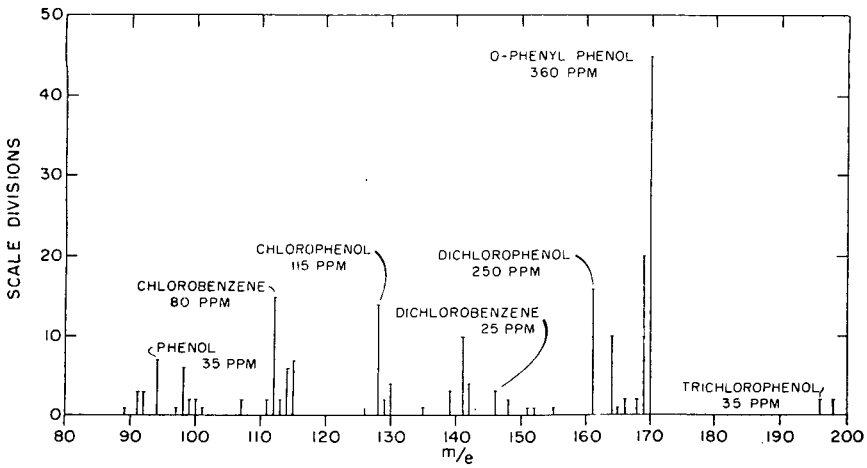


Figure 1. Mass spectrum of a benzene extract of phenol waste water

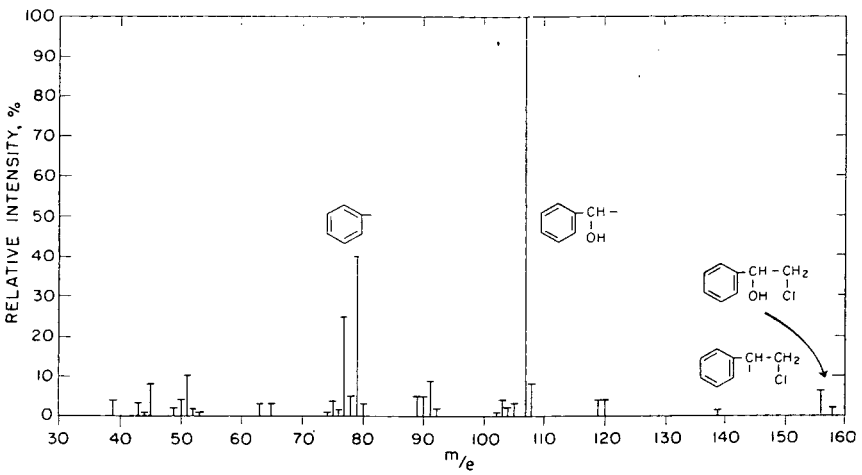


Figure 2. Mass spectrum of styrene chlorohydrin

$S_{107}/S_{Tol} = 0.65$

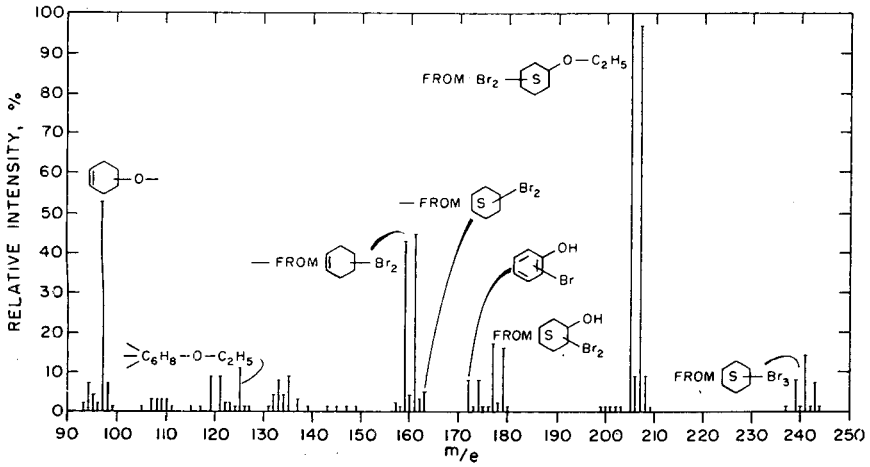
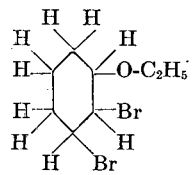


Figure 3. Mass spectrum of crude 2,3-dibromocyclohexyl ethyl ether



the bromination of carbon tetrachloride. Accuracies obtainable are comparable with those on more volatile chloromethanes and -ethanes (±0.3 mole %) (*1*).

Sample decomposition or interaction has caused little trouble at the elevated temperatures in the inlet system. However, quantitative analysis is sometimes possible even when this occurs. Mono- and diethanolamine (Table V) show only small peaks at masses higher than their molecular weights when the inlet is at 200° C. However, triethanolamine, molecular weight 149, gives a large unexplained peak at *m/e* 174 which, though evidently arising from intermolecular reaction, can be used to determine the amount of triethanolamine present. Synthetic blends show that the height of the 174 peak from this mixture is not strictly proportional to concentration of triethanolamine, but by plotting this deviation accurate results can be obtained.

QUALITATIVE ANALYSIS

Applications of the heated-inlet mass spectrometers in qualitative analysis have covered almost the whole range of organic chemistry. Identifications are initially attempted by comparison with reference spectra, as the mass spectrum of a compound is a highly individual characteristic. A file of the spectra of over 2500 different compounds has been collected in this laboratory. Rapid searching of the file is accomplished using IBM punched cards coded with the significant peaks of the individual spectra (*24*).

The mass spectrum obtained from a benzene extract of phenol waste water (Figure 1) shows the contaminants identified from comparison with standard spectra, and the amounts calculated in the original waste water. Similar identification of atmospheric pollutants has been described (*25*).

Even when reference spectra are not available, much unique and useful structural information about an unknown compound is indicated in its mass spectrum, as has been pointed out by Rock (*30*). The bombardment of the sample molecules with electrons produces both molecular and fragment ions which are displayed in the mass spectrum. The electrons used are of sufficient energy to ionize any molecule, so that all components of a mixture will contribute to the spectrum of the mixture. Thus only compounds of insufficient volatility to enter the ion source will fail to register on the spectrum. Compounds undergoing chemical reaction

Table V. Mass Spectra of Ethanolamines

m/e	Mono-	Di-	Tri-	Ion
30	980	28.0	29	$-CH_2NH_2$
45	2.92	11.7	22	$-C_2H_4OH$
56	2.80	43	69	
61	100	0.56	6.3	$HOC_2H_4NH_2$
74	0.20	100	167	$-CH_2NHC_2H_4OH$
105	0.02	1.92	3.9	$(HOC_2H_4)_2NH$
130	0.4	0.34	43	
149	...	0.07	0.8	$(HOC_2H_4)_3N$
174	...	0.09	100	
S/S_{Tol}	0.087	0.47	0.067	

or thermal degradation will register as the spectra of the resulting products.

Many compounds give an appreciable peak corresponding to the ionized molecule. The mass of this ion as shown by the spectrum will be an exact indication of the molecular weight. If the molecule contains an atom having, say, two stable isotopes, then two separate peaks will be observed at the molecular weights corresponding to molecules containing each isotope separately. Thus the mass spectrum of hydrogen chloride, nominal molecular weight 36.5, shows large peaks at mass 36.0 and 38.0 in the ratio of 3 to 1, the natural abundance ratio of chlorine-35 and chlorine-37. Methylene chloride, CH_2Cl_2, shows large peaks at 84, 86, and 88 in the ratio of 9:6:1, corresponding to molecules containing two chlorine-35, one chlorine-35 and chlorine-37, and two chlorine-37 atoms. The existence of multiple stable isotopes for most elements thus often makes possible the determination of the empirical formula in addition to the molecular weight. If the mass of the ion can be determined with sufficient accuracy, this alone will determine the empirical formula (*2*).

Table VI. Analysis of Chlorobenzene Decomposition Products

Mass	No. of Cl Atoms	Tentative Identification	Run A, Approx. %	Run C, Approx. %
296	6	Hexachlorotoluene	2	
290	4	Tetrachlorobiphenyl	..	1
288	6	Hexachlorocyclohexane	1	0.1
282	6	Hexachlorobenzene	3	0.1
262	5	Pentachlorotoluene	10	0.03
256	3	Trichlorobiphenyl	..	1
254	5	Pentachlorocyclohexane	4	1
248	5	Pentachlorobenzene[a]	50	0.1
228	4	Tetrachlorotoluene	1	1
222	2	Dichlorobiphenyl	1	2
220	4	Tetrachlorocyclohexane	2	3
214	4	Tetrachlorobenzene[a]	<0.1	32
202	1	Chloromethylbiphenyl	..	0.4
188	1	Chlorobiphenyl	0.5	3
184	0	Unidentified	10	...
180	3	Trichlorobenzene[a]	<0.1	48
168	0	Methylbiphenyl	1	3
154	0	Biphenyl[a]		4

[a] Available standard indicates this structure highly probable.

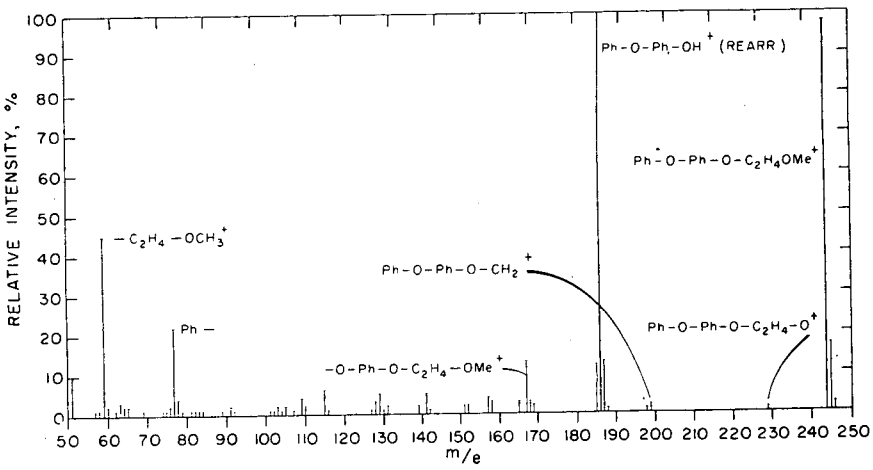
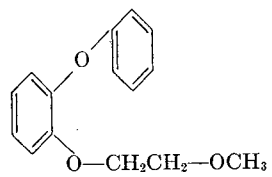


Figure 4. Mass spectrum of phenoxy-β-methoxyphenetole



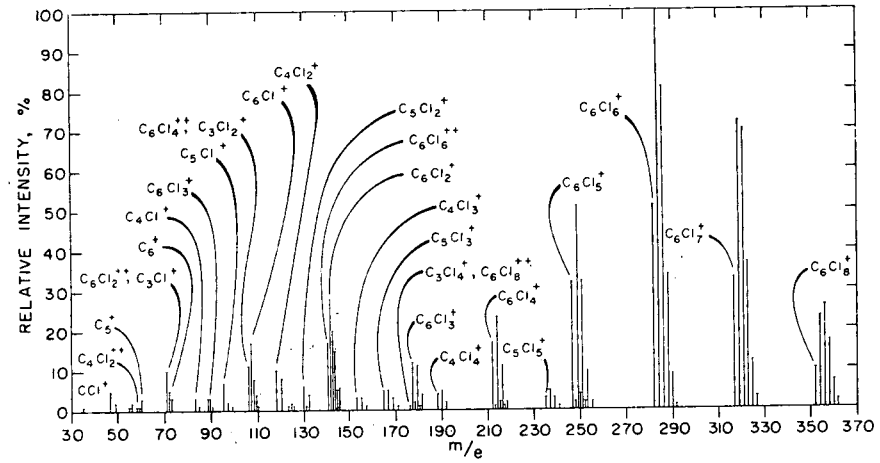


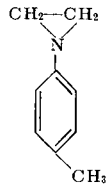
Figure 5. Mass spectrum of octachloro-1,3,5-hexatriene

$S_{284}/S_{Tol} = 0.11$

$CCl_2{=}CCl{-}CCl{=}CCl{-}CCl{=}CCl_2$

The analysis of two samples obtained from the degradation of chlorobenzenes is shown in Table VI. Only four components were identified for which standard mass spectra were available, but strong indications were obtained for the other compounds from the molecular weights and number of chlorine atoms. Thus peaks at masses 282, 284, and 286 in the ratios of 1.00: 1.95:1.53, respectively, indicate a compound of molecular weight 282 (using the most abundant isotopes) containing six chlorine atoms. Mass spectral evidence and chemical supposition suggested hexachlorobenzene as the compound, which was later confirmed when a spectrum of a pure sample was obtained. The mass spectrometer can usually resolve much more complex mixtures than most general analytical methods because of the large number of discrete peaks possible in the spectrum.

Table VII. Mass Spectrum of *N*-*p*-Tolylethylenimine



m/e	Relative Intensity	Ion	*m/e*	Relative Intensity	Ion
64	3.6		104	8.7	
64.5	1.2		105	220	CH_3—Ph—N<
65	27.0		106	23.1	
65.5	10.8		118	33.2	CH_3—Ph—N—CH<
66	3.7		119	3.9	
66.5	2.9	CH_3—Ph—N=$(CH_2)_2$$^{++}$	120	22.7	CH_3—Ph—N(CH_3)— (imp.)
76	3.1		132	21.8	
77	22.9	Ph—	133	100	CH_3—Ph—N=$(CH_2)_2$
78	8.8		134	11.1	
79	18.6		169	3.28	CH_3—Ph—N(CH_3)—CH_2Cl (imp.)
90	6.2		171	1.11	
91	85.0	CH_3—Ph—	213	1.10	CH_3—Ph—N(CH_3)—CH_2Br (imp.)
92	7.8		215	1.07	
			S_{133}/S_{Tol}	0.34	

The spectrum of a purified sample of *N*-*p*-tolylethylenimine (Table VII) shows anomalous peaks at *m/e* 169, 171, 213, and 215. The first two indicate a molecule containing one chlorine atom, and so can well be the addition of hydrogen chloride (mass 36) to the imine (mass 133). In the same way *m/e* 213 and 215 may be the hydrogen bromide adduct (the natural abundance ratio of the 79 and 81 bromine isotopes is almost 1 to 1). The appreciable mass 120 fragment can arise from the imine only by an unusual rearrangement. Determination of the rate of decay of the *m/e* 120 peak caused by effusion through the mass spectrometer leak (*11*) shows that it arises from compound(s) of much higher molecular weight than the imine. The lack of a peak at mass 122 shows that this 120 ion contains no chlorine or bromine. Thus it is probably the fragment formed by loss of —CH_2X from the suggested impurities.

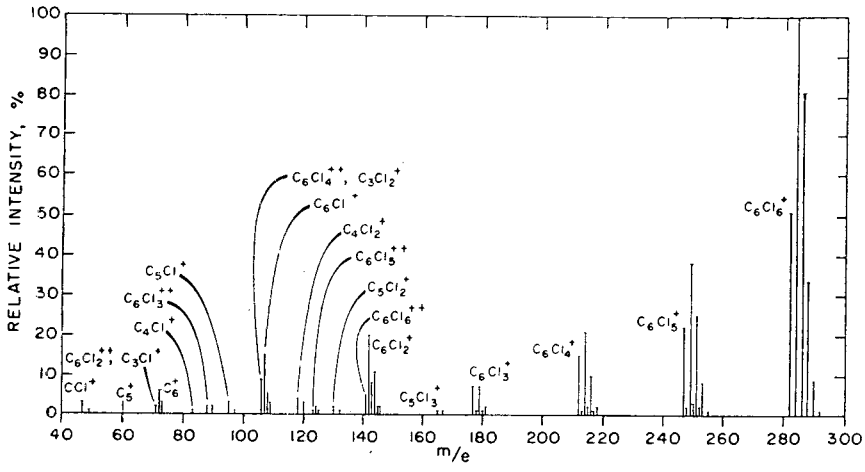
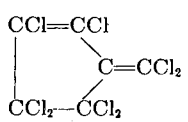


Figure 6. Mass spectrum of octachloro-3-methylenecyclopentene

S_{284}/S_{Tol} = **0.17**



The detection of impurities of high chemical similarity in purified samples is especially difficult by methods based on the chemical properties of a compound. The spectrum of the isopropyl ester of 2,4-dichlorophenoxyacetic acid (Table VIII) shows the presence of a compound of molecular weight greater by 14 mass units, or CH_2. This would correspond to the butyl ester in roughly 1% concentration. In the same way the *m/e* 228 peak is probably the monochloro-ester.

In general, geometric isomers and compounds differing only in the position of substitution on an aromatic ring give very similar mass spectra. There is some steric effect with large groups cis or ortho to each other. However, isomers differing in position of substitution or branching on a chain usually give large spectral differences.

MOLECULAR DISSOCIATION BY ELECTRON BOMBARDMENT

In the mass spectrometer ion source, electron bombardment is thought (*31*) to give first the excited molecular ion. This may then decompose by cleavage of some bond to produce two fragments of the molecule, one of which will retain the positive charge. Further degradation of these products can then take place in a similar manner. The positive ions thus produced can often establish the presence or absence of particular structural pieces of the molecule. Relative quantities of these pieces are an indication of the strength and chemical nature of the bonds uniting them to the rest of the molecule. In general, the probability of cleavage of a particular bond is related both to the chemical lability of the bond and the thermodynamic stability of the fragments that are thus formed. The further papers in this series are planned as detailed studies of the modes of fragmentation of various classes of chemical compounds.

Aliphatic hydrocarbons show greater bond cleavage at the more highly branched points on the carbon chain. Long unbranched chains yield their most abundant ions in the C_2 to C_5 range. Unsaturation in a molecule usually decreases the amount of fragmentation. Vinylic bonds are strengthened, while allylic bonds are weakened. Aromatic and other resonance compounds are highly stabilized, as would also be expected from chemical experience. Bonds alpha to an aromatic ring are strengthened, while those beta are, in general, favored points for cleavage.

The mass spectrum of styrene chlorohydrin (Figure 2) shows its largest peaks through such a beta bond cleavage. Here the structure of the chemically possible isomer, α-chloro-β-hydroxyethyl-

benzene, can be ruled out without reference standards, as it should give large C_6H_5CHCl— and $C_6H_5CHCH_2OH$— ion fragments.

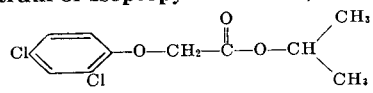
Amines, alcohols, ethers, mercaptans, and other electron-releasing groups typically cause cleavage at the carbon-carbon bond beta to this group. Aliphatic amines are especially specific in this regard, so that the most abundant ion in their spectra is usually the fragment from this rupture which contains the nitrogen atom. If there is more than one beta bond, cleavage of the one holding the largest group is favored. Unsaturation on the α-carbon atom can override this tendency, and alpha substitution usually causes major ion fragments which are not easily explained by simple bond cleavages.

The isomers, 1-methoxy-2-propanol and 2-methoxy-1-propanol (Table IX), contain a carbon-carbon bond that is beta to both the alcohol and ether groups. The isomers can be distinguished readily, as cleavage of this bond gives the largest peaks in the spectra: $CH_3CH(OH)$— and CH_3OCH_2— (both mass 45) in the 1-methoxy-2-propanol, and $CH_3CH(OCH_3)$— (mass 59) and $—CH_2OH$ (mass 31) in 2-methoxy-1-propanol.

Halogen, nitro, and most all carbonyl-type groups tend to weaken the alpha bond for cleavage by electron impact. This does not apparently extend to all electron-attracting groups, however, as some, like nitriles, tend to break at the beta bond. The nitro, carbonyl, and other unsaturated groups are complicated by rearrangement tendencies, as described later. With halogenated molecules, fragmentation increases as the halogen atom is made successively iodine, bromine, chlorine, and fluorine. Loss of a halogen atom with formation of an abundant organic ion is favored for bromo and iodo hydrocarbons, though the chloro and especially the fluoro compounds lose a hydrogen atom with the halogen in many cases. When a neighboring point in the molecule is weakened, as by chain branching or an aromatic group, the inductive effect of the halogen usually enhances this cleavage instead of showing the rupture of the carbon-halogen bond.

The probable impurities causing the anomalous peaks in the spectrum of a crude sample of 2,3-dibromocyclohexyl ethyl ether (Figure 3) can be

Table VIII. Mass Spectrum of Isopropyl Ester of 2,4-Dichlorophenoxyacetic Acid



m/e	Relative Intensity	Ion	m/e	Relative Intensity	Ion
161	10.9	Cl_2—Ph—O—	227	1.9	Cl—Ph—$OCH_2COOC_3H_7$
162	79.4	Cl_2—Ph—OH (rearr.)	228	1.5	Cl—Ph—$OCH_2COOC_3H_7$ (imp.)
163	12.4		229	0.7	
164	50.0		230	0.5	
175	145.2	Cl_2—Ph—OCH_2—	233	0.39	Cl_2—Ph—OCH_2COOCH_2—
176	27.4	Cl_2—Ph—OCH_3 (rearr.)	235	0.24	
177	95.8		247	0.15	Cl_2—Ph—$OCH_2COOC_2H_4$—
178	17.3		249	0.09	
185	44.8	Cl—Ph—OCH_2COOH	262	100	Cl_2—Ph—$OCH_2COOC_3H_7$
187	14.8		264	65.0	
219	9.5	Cl_2—Ph—OCH_2COO—	276	1.0	Cl_2—Ph—$OCH_2COOC_4H_9$ (imp.)
220	35.9	Cl_2—Ph—OCH_2COOH (rearr.)	278	0.6	
221	9.4		S_{262}/S_{Tol}	0.095	
222	23.3				

Table IX. Mass Spectra of Isomeric Methoxypropanols

Mass	Ion	$CH_3CHOHCH_2OCH_3$	$CH_3CH(OCH_3)CH_2OH$
31	$—CH_2OH$, $—OCH_3$	10.6	27.4
45	CH_3CHOH—, CH_3OCH_2—	100	5.9
47	C_2H_7O— (rearr.) ?	30.0	0.06
59	$CH_3CH(OCH_3)$—, $CH_3(C_2H_3OH)$—	1.9	100
75	—$C_2H_3(OH)(OCH_3)$, $CH_3C_2H_3(OH)O$—	4.1	1.5
90	$CH_3C_2H_3(OH)(OCH_3)$	0.7	0.6
S_B/S_{Tol}		2.3	2.0

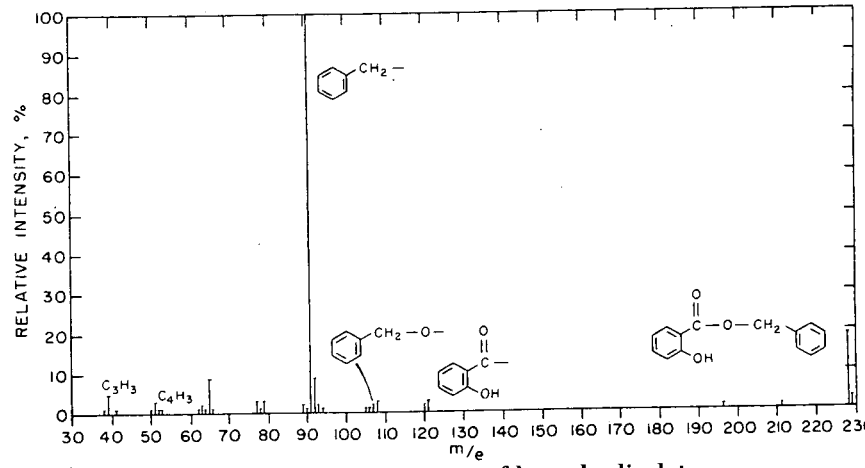


Figure 7. Mass spectrum of benzyl salicylate

S_{91}/S_{Tol} = 0.56

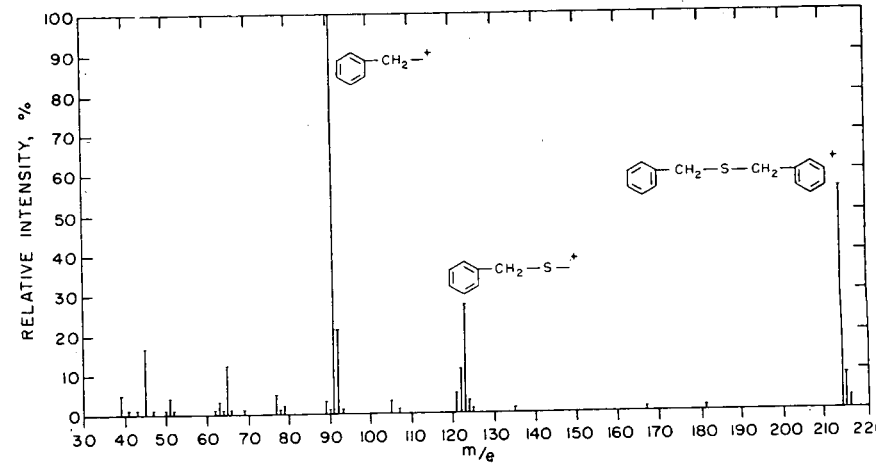


Figure 8. Mass spectrum of benzyl sulfide

S_{91}/S_{Tol} = 0.76

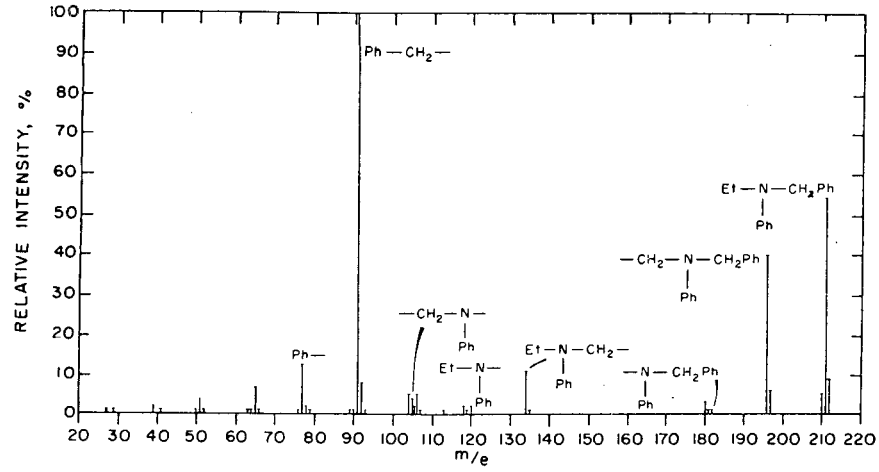
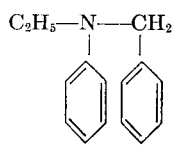


Figure 9. Mass spectrum of ethylbenzylaniline

$S_{91}/S_{Tol} = 1.1$



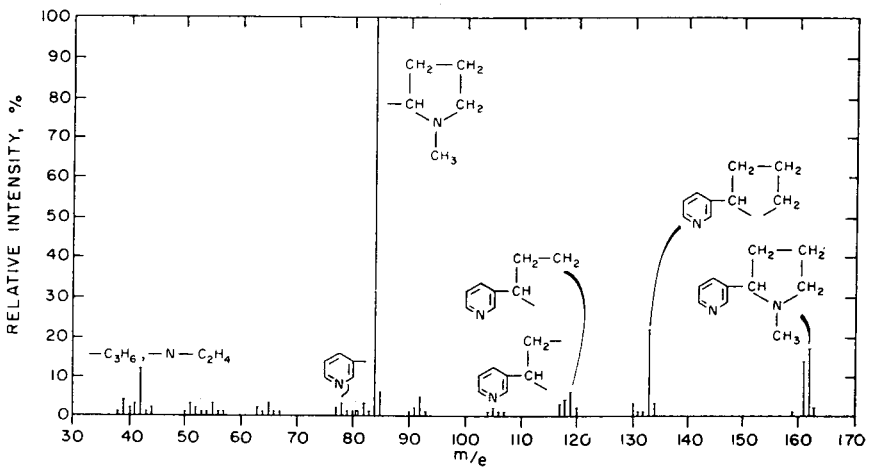


Figure 10. Mass spectrum of nicotine

$S_{84}/S_{Tol} = 0.46$

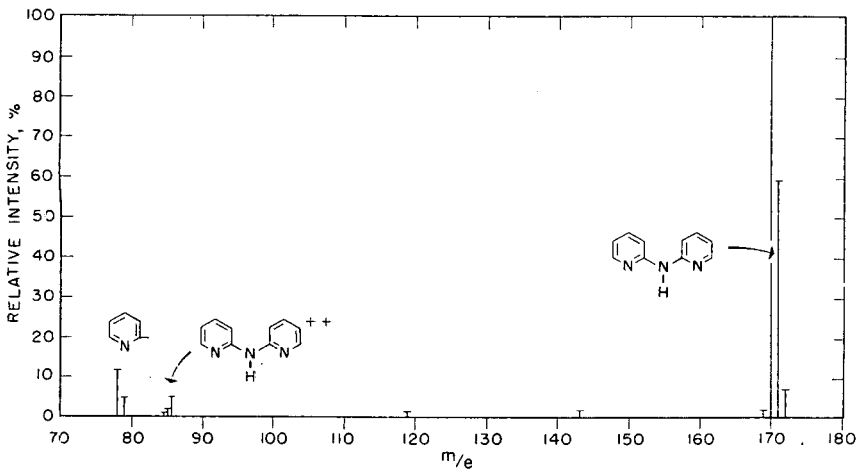


Figure 11. Mass spectrum of 2,2′-dipyridylamine

$S_{170}/S_{Tol} = 1.0$

deduced assuming such behavior of halogen compounds. The characteristic 1 to 1 natural abundance ratio of the bromine isotopes of masses 79 and 81 shows the number of these atoms in the various fragments. The first C—Br bond is cleaved very readily, giving thus the largest peaks (*m/e* 205 and 207) from the dibromocyclohexyl ethyl ether. Similar fragmentation of the postulated compounds shown would give the anomalous peaks indicated. The loss of the bromine atom from bromophenol is comparatively small, as this involves rupture of the stable α-phenyl bond. Standards for this compound and two of the others confirm their identifications.

MOLECULAR REARRANGEMENTS IN MASS SPECTRA

The main difficulty encountered in structure assignments from the ion fragments in the mass spectrum is that sometimes fragments occur that cannot arise from simple cleavage of bonds in the molecule. Several sizable peaks of this kind (*m/e* 220, 176, and 162) occurred in the spectrum of the isopropyl ester of 2,4-D (Table VIII). The largest peak in the cracking pattern of phenoxy-β-methoxyphenetole, (Figure 4) must be due to such a rearrangement. The mass 186 peak can be shown not to be due to an impurity such as phenoxyphenol by its rate of decay during effusion (*11*), by its high appearance potential (*34*), by comparison with the reference spectrum of pure phenoxyphenol, by additional sample purification, or by independent purity determination (as freezing point depression) on the sample.

Much of the analytical disadvantage incurred by these rearrangements would be removed if they could be predicted from the molecular structure of the compound. No such generalizations have been reported, though evidence has been collected for various mechanisms of rearrangements in hydrocarbons (*13, 17, 20, 21, 33*). Rearrangements appear to occur so as to give more stable products from a particular bond cleavage or cleavages. For example, production of smaller fragments from a cyclic molecule requires breaking two bonds, so that an ion thus produced contains an odd number of electrons. Intra-molecular hydrogen migration when breaking these bonds gives a more stable "even-electron" ion (*13, 21*). Similarly, the large amount of $CClF_2^+$ ion in the mass spectrum of $CClF{=}CF_2$ can be explained by halo-

gen migration to stabilize the "odd-electron" ion formed by cleavage of the double bond. Rearrangements producing odd-electron ions, such as mass 60 from butyric and higher acids (*16*), can be explained (*15*) by the formation of a stable, "even-electron" neutral fragment by the intramolecular hydrogen atom migration. Thus appreciable rearrangement usually occurs in a compound that bears a hydrogen at least two carbon atoms (or often carbon and another atom) removed from an "allylic" bond (thought of here as a bond beta to double bonds such as carbonyl, nitrile, and phenyl also). The presence of oxygen and other hetero atoms near the allylic bond in the fragment to which the hydrogen migrates helps also. Thus the 186 peak in the phenoxy-β-methoxyphenetole is formed from hydrogen migration to the aromatic fragment after cleavage of the allylic oxygen-carbon bond. Similarly, the largest peaks in the spectra of such diverse compounds as butyric acid (*16*), *n*-butyraldehyde, *n*-butyronitrile, *n*-butyramide, phenetole, and dibutyl phthalate can be accounted for by this mechanism. No appreciable amount of this rearrangement takes place in the spectra of propionic acid, propionaldehyde, propionitrile, propionamide, anisole, or dimethyl phthalate, as there is no hydrogen two chain atoms removed from the allylic bond. Both steric factors and the formation of the stable even-electron ethylene fragment are thought to contribute to this requirement.

Many of the major peaks of triethyl phosphate (Table X) must be due to the rearrangement of more than one hydrogen atom, giving an even-electron ion as the stable product. The presence of extra nonbonding electrons on the phosphorus atom, as well as the probable stability of the rearranged ions, may cause this. Similar "double rearrangements" are shown by cyclohexylacetic acid and isocrotonic acid (though not appreciably by crotonic acid).

Correlations appear possible for other rearrangements, such as the large $C_2Cl_2F_3$— ion in the spectrum of $CCl_3—\overset{O}{\overset{\|}{C}}—CF_3$, C_7H_8— in the spectrum of $C_6H_5CH_2OCH_2C_6H_5$, CH_2F— in the spectrum of CH_2Cl-CF_3, CH_5O— in the spectrum of $(CH_3)_2CHCH_2OH$, C_7H_8O— in the spectrum of $C_6H_5CH_2O\overset{O}{\overset{\|}{C}}CH_3$, and

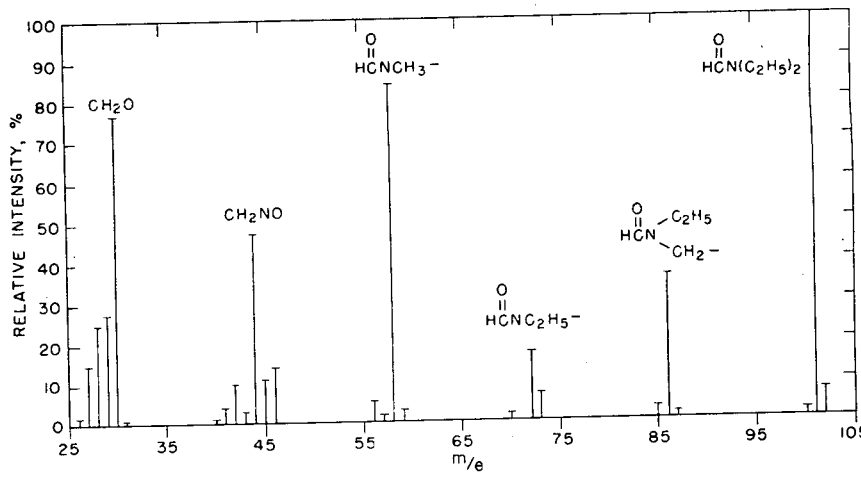


Figure 12. Mass spectrum of diethylformamide

S_{101}/S_{Tol} = 0.28

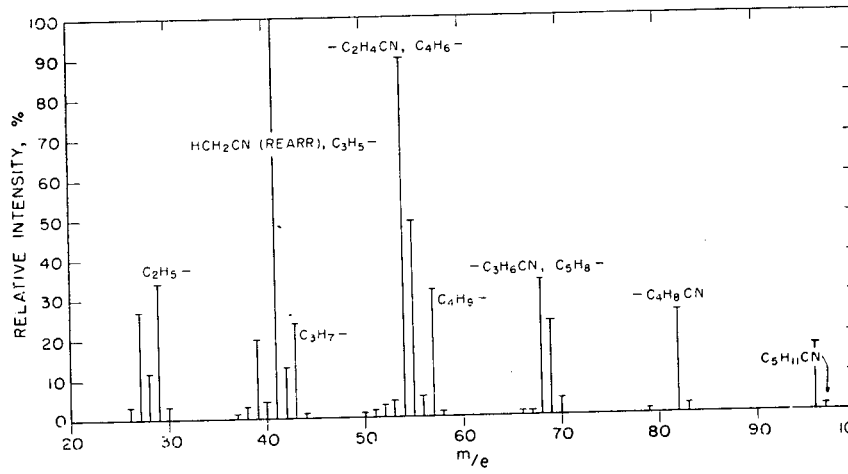


Figure 13. Mass spectrum of *n*-capronitrile

S_{41}/S_{Tol} = 0.44

$CH_3CH_2CH_2CH_2CH_2CN$

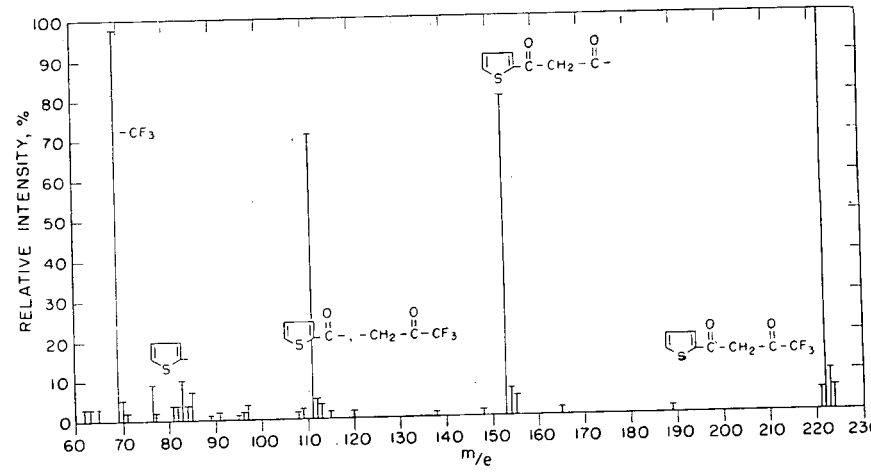
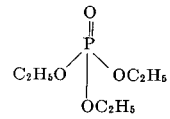


Figure 14. Mass spectrum of thenoyltrifluoroacetone

S_{222}/S_{Tol} = 0.14

Table X. Mass Spectrum of Triethyl Phosphate



m/e	%	Probable Ion	m/e	%	Probably Ion
29	19.5	C_2H_5	125	18.4	$C_2H_6O_4P$
45	14.6	C_2H_5O	126	5.9	$C_2H_7O_4P$
81	43	H_2O_3P	127	56	$C_2H_8O_4P$
82	31	H_3O_3P	137	11.4	$C_4H_{10}O_3P$
83	11.1	H_4O_3P	138	10.9	$C_4H_{11}O_3P$
97	0.37	H_2O_4P	139	10.4	$C_4H_{12}O_3P$
98	0.24	H_3O_4P	153	5.2	$C_4H_{10}O_4P$
99	93	H_4O_4P	154	2.78	$C_4H_{11}O_4P$
100	0.23	(Isotope)	155	100	$C_4H_{12}O_4P$
109	41	$C_2H_6O_3P$	167	4.2	$C_5H_{12}O_4P$
110	8.4	$C_2H_7O_3P$	181	3.3	$C_6H_{14}O_4P$
111	15.5	$C_2H_8O_3P$	182	13.6	$C_6H_{15}O_4P$
S_{155}/S_{Tol}	0.43				

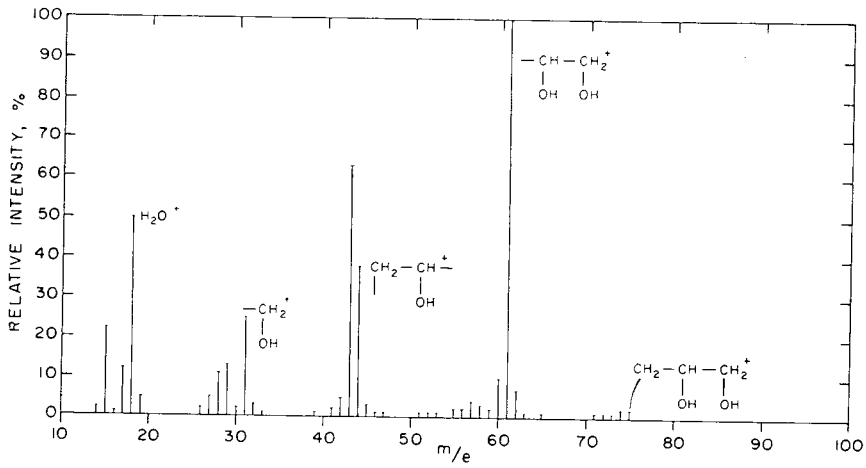
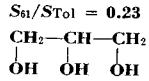


Figure 15. Mass spectrum of glycerol

S_{61}/S_{Tol} = **0.23**



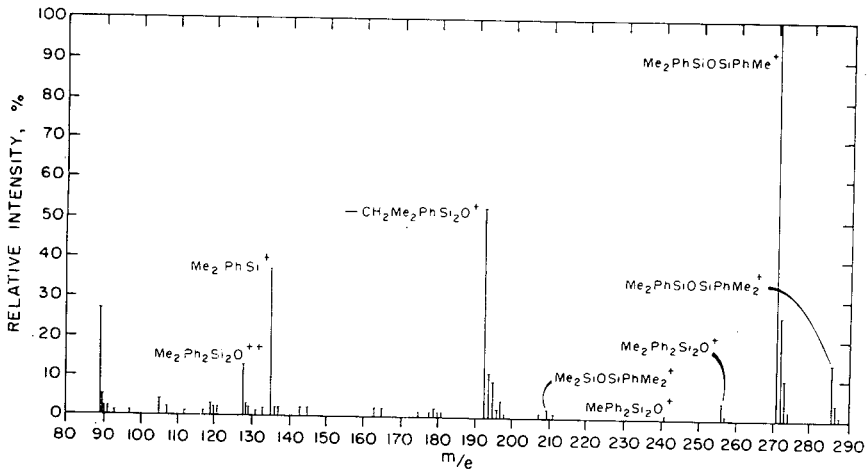
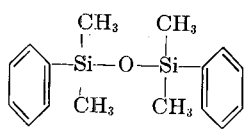


Figure 16. Mass spectrum of tetramethyldiphenyldisiloxane

S_{127}/S_{Tol} = **0.52**



$C_3H_6O_2$— in the spectrum of C_2H_5OCC-OC_2H_5. Such correlations should greatly reduce this major drawback to the use of the mass spectrometer in determinations of molecular structure.

DIVERSITY OF ANALYZABLE COMPOUNDS

The heated-inlet mass spectrometer has been applied to a wide range of chemical compounds in this laboratory. Some random examples are shown to illustrate the information available in the mass spectra of various chemical types. The mass spectrum of octachloro-1,3-5-hexatriene (Figure 5) shows a sizable molecular ion, $C_6Cl_8^+$, because of the resonance stabilization of the molecule and vinylic attachment of the chlorine. However in the case of the isomeric octachloro - 3 - methylenecyclopentene (Figure 6), there is no molecular ion, and there is a large loss of the allylic chlorine atoms. The conjugated and cyclic carbon skeleton shows little degradation. Another isomer, octachlorohexene-5-yne, shows a little less chlorine loss, but more carbon-carbon scission than the methylenecyclopentene.

Benzyl salicylate (Figure 7) gives the parent and benzyl ion as the major peaks in its spectrum.

Benzyl sulfide (Figure 8) also illustrates the ease of breaking bonds beta to the phenyl ring in preference to the alpha bonds. This overshadows the tendency for cleavage at bonds beta to a sulfur atom.

Ethylbenzylaniline (Figure 9) also gives its largest peaks as the benzyl and molecular ions. The tendency in amine compounds for rupture of bonds beta to the nitrogen atom accounts for the significant ions at *m/e* 196 and 134, and possibly 77.

Table XI. Mass Spectrum of Perfluorokerosine

m/e	%	Ion	m/e	%	Ion	m/e	%	Ion	m/e	%	Ion
69	100	CF_3	281	5.2	C_6F_{11}	381	2.3	C_8F_{15}	486	0.08	$C_{12}F_{18}$
100	6.3	C_2F_4	286	0.30	C_8F_{10}	386	0.09	$C_{10}F_{14}$	493	1.4	$C_{11}F_{19}$
119	25	C_2F_5	293	5.7	C_7F_{11}	393	2.3	C_9F_{15}	505	0.45	$C_{12}F_{19}$
131	25	C_3F_5	305	1.2	C_8F_{11}	405	1.4	$C_{10}F_{15}$	512	0.11	$C_{11}F_{20}$
169	13	C_3F_7	312	0.12	C_7F_{12}	412	0.07	C_9F_{16}	517	0.13	$C_{13}F_{19}$
181	17	C_4F_7	317	0.47	C_9F_{11}	417	0.31	$C_{11}F_{15}$	524	0.04	$C_{12}F_{20}$
219	5.0	C_4F_9	319	1.0	C_6F_{13}	419	0.09	C_8F_{17}	531	0.66	$C_{11}F_{21}$
224	0.34	C_6F_8	324	0.23	C_8F_{12}	424	0.30	$C_{10}F_{16}$	536	0.06	$C_{13}F_{20}$
231	7.8	C_5F_9	331	3.4	C_7F_{13}	431	1.2	C_9F_{17}	543	0.77	$C_{12}F_{21}$
236	0.63	C_7F_8	336	0.11	C_9F_{12}	436	0.09	$C_{11}F_{16}$	555	0.24	$C_{13}F_{21}$
243	5.4	C_6F_9	343	4.1	C_8F_{13}	443	1.8	$C_{10}F_{17}$	562	0.06	$C_{12}F_{22}$
255	1.9	C_7F_9	348	0.04	$C_{10}F_{12}$	448	0.03	$C_{12}F_{16}$	567	0.06	$C_{14}F_{21}$
262	0.23	C_8F_{10}	355	1.1	C_9F_{13}	455	0.87	$C_{11}F_{17}$	574	0.02	$C_{13}F_{22}$
263	0.18	C_6HF_{10}	362	0.12	C_8F_{14}	462	0.06	$C_{10}F_{18}$	581	0.33	$C_{12}F_{23}$
268	0.68	C_8F_9	367	0.38	$C_{10}F_{13}$	467	0.23	$C_{12}F_{17}$	593	0.24	$C_{13}F_{23}$
269	2.4	C_5F_{11}	369	0.34	C_7F_{15}	469	0.05	C_9F_{19}	605	0.08	$C_{14}F_{23}$
274	0.27	C_7F_{10}	374	0.14	C_9F_{14}	474	0.16	$C_{11}F_{18}$	617	0.04	$C_{15}F_{23}$
275	0.19	C_7HF_{10}	379	0.09	$C_{11}F_{13}$	481	0.88	$C_{10}F_{19}$	631	0.08	$C_{13}F_{25}$
									643	0.05	$C_{14}F_{25}$

S_{69}/S_{Tol} 0.34

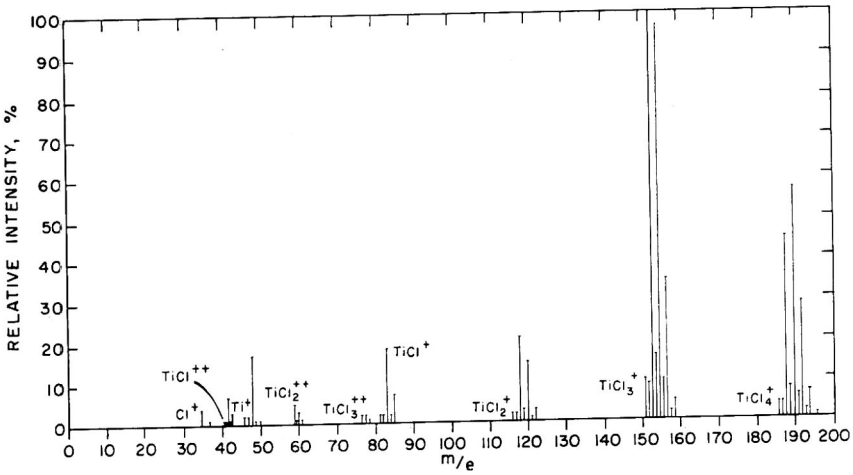


Figure 17. Mass spectrum of titanium tetrachloride

S_{153}/S_{Tol} = 0.23

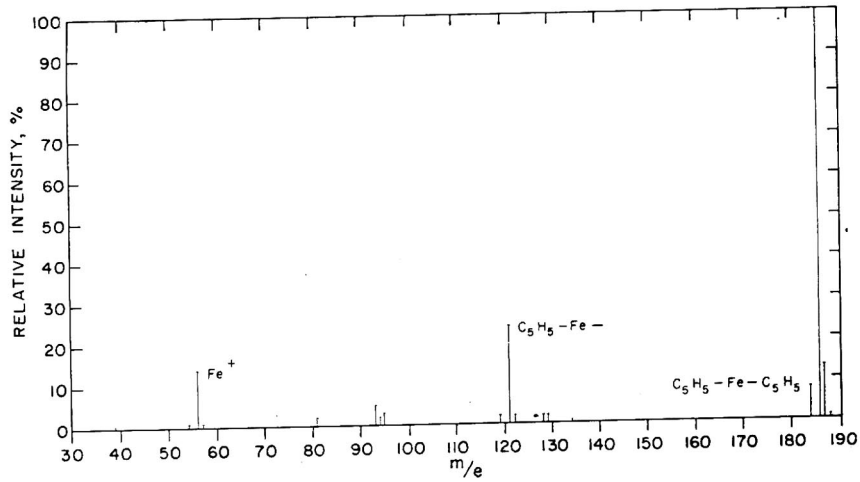
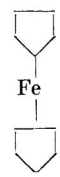


Figure 18. Mass spectrum of ferrocene

S_{186}/S_{Tol} = 0.93



A similar cleavage of the bond beta to an amino nitrogen atom gives the largest peak in the spectrum of nicotine (Figure 10). This bond is also weakened by its attachment to the saturated ring. Though the fragmentation of this ring can be closely followed in the spectra, no appreciable peaks are found from breakdown of the resonance-stabilized pyridine nucleus.

With two pyridine nuclei, 2,2′-dipyridylamine (Figure 11) is so stabilized that it shows little fragmentation. The large loss of one hydrogen atom is very probably from cleavage of the hydrogen-nitrogen bond that is beta to both rings.

Diethylformamide (Figure 12) shows stepwise loss of methyl and methylene groups, similar to aliphatic hydrocarbons. The large 30, 44, and 58 peaks are probably from rearrangements of the molecule to the empirical formulas shown.

The largest peak in the spectrum of *n*-capronitrile (Figure 13) at *m/e* 41 can be explained by the hydrogen rearrangement after beta-bond cleavage described previously. This can also be due to a $C_3H_5^+$ ion. It should be possible to determine the relative amounts of these two fragments at high resolution, owing to their slight differences in mass (*2*).

Thenoyltrifluoroacetone (Figure 14) shows the favored cleavage of bonds to carbonyl groups. Of the two possible fragments accounting for the 111 peak, the thenoyl one is indicated by the 113 peak. Sulfur has 32, 33, and 34 isotopes in a natural abundance ratio of 95.1:0.7:4.2. Absolute mass determination (*2*) could also differentiate this.

Both carbon-carbon bonds in glycerol (Figure 15) are beta to two hydroxyl groups, leading to a large amount of fragmentation and lack of molecular ion.

The largest peak in the spectrum of tetramethyldiphenyldisiloxane (Figure 16) is formed from scission of carbon-silicon bonds beta to the phenyl groups. The number of silicon atoms in a particular ion is readily determined from the characteristic abundances of the mass 28, 29, and 30 silicon isotopes.

Volatile metal halides such as titanium tetrachloride (Figure 17) are becoming common industrially. The overlapping isotopes of titanium and chlorine make a large family of peaks for each ion formula. There is an appreciable parent peak, in contrast to carbon tetrachloride. This is probably due to greater ease of ionization caused by loss of nonbonding electrons from the titanium atom.

Ferrocene (Figure 18) is an interesting metallo-organic compound whose high stability lies in the conjugation of the cyclopentadiene rings through bonding to the iron atom in the middle of the "sandwich." The almost total lack of fragmentation of the rings in the spectrum gives further proof of this concept.

The partial spectrum of perfluorokerosine (Table XI) shows how an apparently complex spectrum can be elucidated assuming simple bond cleavages. This material, obtained from the Organic Chemicals Department, E. I. du Pont de Nemours & Co., is very useful as an internal standard for absolute determination of the mass of a particular peak in another sample, because of both its multiplicity of identifiable peaks and its high volatility.

CONCLUSIONS

The mass spectrometer provides an abundance of useful and unique information about a compound or mixture. Because of its broad applicability to all types of chemicals, it seems surprising that most emphasis has been on petroleum compounds. Of the 10,000 samples per year analyzed by mass spectrometry in this laboratory, the great majority are of a chemical nature. It is hoped that this paper has helped show that the mass spectrometer is a general tool for both qualitative and quantitative analysis in chemical research and production.

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Trace Components by Sorption and Vaporization in Mass Spectrometry

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Sorption and vaporization during introduction of samples to a mass spectrometer often cause changes in composition and thereby interfere with quantitative analysis. An elution technique in a brass sample-introduction system converts this liability into an asset by making the changes readily apparent. The changes in composition are used to detect, identify, and quantitatively determine minor components that differ from the principal component in sorptive behavior or volatility. This technique has proved particularly useful in evaluating the spectra of pure compounds for use as reference spectra.

WHEN samples are introduced to the mass spectrometer, partial separation of components by sorption and/or vaporization is a common problem (*6, 15*). The problem is intensified with compounds that are heavily sorbed and slightly volatile; thus, it is more serious with liquids and solids than with gases. Sorption is particularly troublesome in systems constructed of certain metals; it is much greater on brass, for instance, than on glass. Selective vaporization is particularly serious for substances with low volatility at the temperature of the inlet system. To minimize sorption and selective vaporization, brass surfaces are usually eliminated and provision is made to heat the inlet system for quantitative analysis of liquids and solids (*7, 10*).

The term "sorption," as used here, includes adsorption on the confining walls, solution in other sorbed organic species, and merely low volatility. Differences in intensity of sorption are readily apparent: cycloalkanes are sorbed more strongly than alkanes; alkylbenzenes more than aliphatic hydrocarbons; styrenes, Tetralins, and indanes more than alkylbenzenes; heavier members of any homologous series more than lighter ones; and polar compounds more than nonpolar.

Sorption and selective vaporization, so troublesome in conventional quantitative analysis, can be converted to distinct assets in other applications and provide information not easily obtainable by other means. The changes in composition that result from sorption or during vaporization can be used to detect, identify, and quantitatively determine minor components that differ from the principal component in sorptive behavior or volatility. This approach has proved particularly useful in evaluating the spectra of pure compounds for use as reference spectra.

EXPERIMENTAL

The instrument used was a Consolidated Model 21-102 mass spectrometer that had been electrically modified to permit scanning to m/e 250 at constant magnet current (*4*). Most liquid samples were admitted from a constant-volume pipet (*2, 12*) through a mercury orifice (*3*). Viscous liquids and solids were vaporized from a test tube at room temperature (*1*). Both the mercury orifice and the test tube led to the expansion volume via a brass valve block (*8*).

Each sample run was followed by one or more water-elution runs. Water was the preferred desorbent because it desorbs many organic compounds and its low molecular weight avoids interference with the peaks from the desorbed material. The inlet system was pumped out, a pipet of water was admitted, and another spectrum was recorded. This spectrum showed those species that had been sorbed and were now desorbed by the water; differences in sorptive behavior usually caused the water-elution spectrum to differ from the original sample spectrum.

When a sample was vaporized from a test tube, the peaks from the more volatile components were magnified in the resulting spectrum. Subsequent reduction of these peaks in the water-elution spectrum enabled easy detection and identification of volatile components present in trace concentrations.

The differences between a sample spectrum and the subsequent water-elution spectrum identified the peaks due wholly or in part to minor components. Furthermore, they established minimum peak contributions due to these components. Analysis of the difference spectrum gave direct information about the molecular structures involved (*9, 13*).

Water-elution runs qualitatively reveal materials remaining in the system. This information, together with experience on difficultly desorbed materials and a careful record of the recent sample history of the instrument, was used in selecting the next sample, in order to avoid masking or interference in critical parts of the spectrum.

RESULTS

Two examples illustrate the detection and identification of trace components in highly purified materials. In the one example, 2-phenyl-3-methylbutane, the impurities were more intensely sorbed than the main component. In the other, 2-methylbenzothiophene, the impurity was more volatile and less intensely sorbed than the main component.

Differences in relative intensities between the sample spectrum and water-elution spectrum of 2-phenyl-3-methylbutane were small but distinguishable:

m/e	Sample	Water Elution
146	0.23	0.3
122	0.01	0.2
105	100.0	100.0

These differences must be due to contaminants. Further, because peaks at 146 and 122 seem to be anomalous in the spectrum of the pure hydrocarbon, these peaks were attributed wholly to contaminants. The molecular weights 146 and 122, the masses of other associated peaks not shown, and the history of the sample suggest 2-phenyl-3-methyl-2-butene and 1-phenylethanol as the most probable contaminants.

2-Methylbenzothiophene is solid at room temperature and was introduced by vaporization from a test tube. The relative intensities in the sample spectrum and water-elution spectrum again were different:

m/e	Sample	Water Elution
147	100.0	100.0
136	0.07	0.03
135	0.18	0.07
134	1.64	0.67

Differences between the spectra revealed benzothiophene, with a molecular weight of 134, as the contaminant; the differences at 135 and 136 are caused by benzothiophene ions that contain heavy isotopes.

Benzene (*8*) and the C_7 and C_8 alkylbenzenes are sorbed only slightly. When these light homologs are present as impurities in heavier alkylbenzenes, they disappear rapidly from the water-elution spectra, as evidenced by the identity of successive spectra. The excess peak height in the sample spectrum is attributed to the impurity and can be used to determine it quantitatively when, as is the case here, insignificant amounts of it are sorbed in the inlet system.

Table I lists the contaminants found by this method in heart

Table I. Trace Components Found in Heart Cuts of Alkylbenzenes

Alkylbenzene	B.P., ° C./Mm. Hg	Contaminants, Volume % Benzene	Toluene	Ethylbenzene
2-Phenyl-2-methylpentane	87/20	0.07	...	...
2-Phenylpentane	92/34	...	...	0.9
3-Phenyl-3-methylhexane	112/20	0.02	...	0.01
3-Phenyl-3-ethylpentane	132/50	0.02	...	...
1-Phenylheptane	145/50	0.05	0.02	0.02

cuts from distillations of several alkylbenzenes. Prolonged distillation at temperatures above 200° C. has been reported to cause significant decomposition of hydrocarbons (*14*). The data given in Table I suggest that slight decomposition may occur even at temperatures well below 200° C.

DISCUSSION

The results reported in Table I illustrate that components, once identified, can be determined if means are available for measuring their contributions to the sample spectrum free of sorption and vaporization effects. In most cases, this condition would require two separate instruments or two parallel inlet systems to one instrument, such that one sample admission is subject to sorption and selective vaporization while the other is free of them. The separations that occur on brass surfaces strongly suggest a crude gas-chromatography column (*5, 11*). This analogy, in turn, suggests that the versatility of the method might be much enhanced by provision for a choice of sorbing media, of eluents, and of operating temperatures.

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Spectrophotometric Method for Quantitative Evaluation of Early Stages of Hydrolyses of Branched Components of Starches by Alpha-Amylases

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A sensitive method has been developed for measurements of the initial stages of the hydrolysis of waxy maize starch by pancreatic amylase from swine. It can be used also under suitable conditions to follow the initial stages of hydrolysis of the branched components of other starches by pancreatic amylase or by other α-amylases. The method is an adaptation of the blue-value technique originally developed for linear components of starches. It makes use of the absorption spectra of the complexes of iodine with the substrate and with its early hydrolysis products. The method makes it possible to determine the relative rates of hydrolysis of dilute solutions of branched components of starches. It is rapid, precise, and convenient for kinetic studies. By calibration of the absorption ratios against the free aldehyde groups formed, it becomes possible to express the velocity of the hydrolysis obtained by the spectrophotometric method in terms of the glucosidic linkages hydrolyzed.

ALTHOUGH other methods have been suggested from time to time, quantitative studies of the action of amylases have, until recently, been based largely upon measurements of the increase in free aldehyde groups resulting from the hydrolysis of 1,4-α-D-glucosidic linkages of the substrate. However, such measurements are not suited to precise quantitative determinations of the initial stages of hydrolyses catalyzed by α-amylases. In the initial stages of their action, α-amylases cause a very large reduction in the average molecular weight of the substrate for each glucosidic linkage hydrolyzed; the free aldehyde groups thus formed on large fragments are difficult to detect or determine by methods developed by the use of lower molecular weight reducing sugars such as glucose and maltose.

In 1943, McCready and Hassid (*8*) reported the blue-value method that is well adapted to the study of the early stages of amylase hydrolyses of linear components of starches. The method is based upon spectrophotometric measurements of complexes of iodine with these substrates and with certain of their hydrolysis products (*6, 8*). This procedure, with certain minor modifications (*3, 7, 10*), has been found useful by other investigators (*7, 10*).

In the method reported here, the blue-value technique has been adapted for the study of the early stages of the hydrolysis of fat-free waxy maize starch (*9, 12*) by crystalline pancreatic amylase from swine (*4*). The procedure permits the precise determination of the course of the early stages of the action of the amylase. The method is especially useful for kinetic studies with dilute solutions of the substrate. It can be employed to determine relative rates of hydrolysis. By calibration of the absorption values against the free aldehyde groups formed, it becomes possible to express the velocity of the hydrolysis obtained by spectrophotometric measurements in terms of the glucosidic linkages hydrolyzed.

Although it was developed for an investigation of the hydrolysis of defatted waxy maize starch by pancreatic amylase from swine, the method can be employed under suitable conditions to follow the hydrolysis of the branched components of other starches by pancreatic amylase or by other α-amylases.

REAGENTS AND APPARATUS

Iodine reagent. Dissolve 3.10 grams of iodine and 31 grams of potassium iodide per liter of distilled water.

Hydrolysis mixture, a solution of defatted branched starch, amylase, and appropriate buffer and salts reacting at 30° C., at 40° C., or at any other appropriate temperature.

Blank solution, identical with the hydrolysis mixture except that the enzyme is omitted.

Iodine starch complex solution. Dissolve 0.05 gram of starch and 0.62 gram of iodine per liter.

The instrument used was a Beckman Model DU spectrophotometer thermostated at 30° C.

PROCEDURE

Ten milliliters of iodine reagent are pipetted into a series of 50-ml. volumetric flasks. To one of the flasks a suitable volume of the blank solution is added to give a final starch concentration, after dilution, equal to 0.05 gram per liter. The solution is diluted to volume when convenient. It is allowed to come to equilibrium for at least 30 minutes in a 30° C. water bath, then absorbance of the complex of iodine with the original substrate (OD_0) is read in a Beckman spectrophotometer at a wave length of 540 mμ. The procedure is repeated at intervals with portions of the hydrolyzate instead of the blank solution. These measurements give the absorbance of the complex of iodine with the hydrolysis products. These absorbances are designated OD_t, where t is the time of hydrolysis.

The absorption ratios (AR_t) then are calculated and plotted versus time of hydrolysis, t.

$$AR_t = \frac{OD_t}{OD_0}$$

The relative rate of reaction is determined from the slope of this curve or from the reciprocal of the time required to reach a given absorption ratio.

RESULTS

Development of Method. Branched components of starches do not absorb iodine readily (*1, 2, 8, 11, 13*). However, the data given in Figures 1 and 2 show that solutions containing as little as 0.005% waxy maize starch can be used as the basis for an analytical method, provided sufficiently large concentrations of iodine also are present.

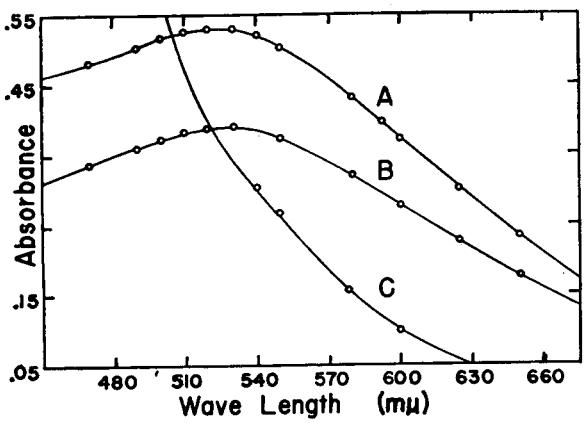

Figure 1. Absorption spectra of complex of iodine with waxy maize starch

A. 0.005% starch in 0.062% iodine, 0.62% potassium iodide
B. 0.005% starch in 0.0186% iodine, 0.186% potassium iodide
C. 0.062% iodine in 0.62% potassium iodide
A and *B* were measured using the appropriate iodine–potassium iodide blank. A water blank was used for *C*.

In Figure 1, the absorption spectra of 0.005% waxy maize starch in 0.062 and 0.018% iodine solutions are shown. These spectra were measured in a Beckman Model DU spectrophotometer thermostated at 30° C., with a 1-cm. cuvette. The appropriate iodine solution was used as the blank. For comparison, the spectrum of a 0.062% iodine solution is included. A broad absorption maximum occurs in the iodine–waxy maize starch spectrum at a wave length of about 525 mμ. However, the spectrum is distorted at lower wave lengths, because the large absorbance of the iodine blank makes a large slit width necessary. For this reason, the absorption was characterized at a wave length of 540 mμ instead of at the maximum.

The effect of iodine concentration on the spectrum of the complex of iodine with waxy maize starch is shown further in Figure 2, where the absorbance at 540 mμ is plotted against iodine concentration. An iodine concentration of 0.062% was chosen for the analytical method.

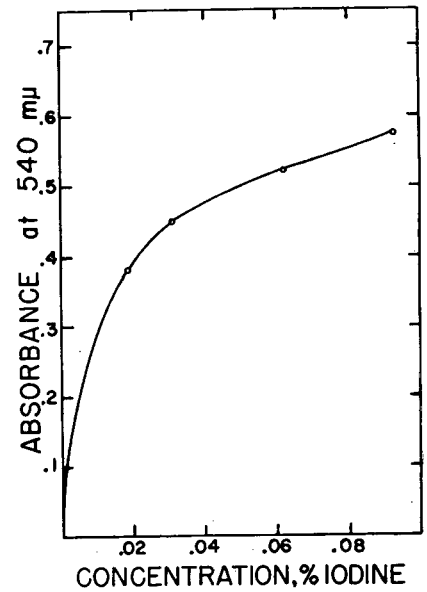

Figure 2. Effect of iodine concentration on absorption spectrum of the complex of iodine with waxy maize starch at 540 mμ

0.005% starch present in each case

Calibration Curve for Conversion of Absorption Ratio to Per Cent Theoretical Glucose. The method as outlined is useful for the determination of relative rates of hydrolysis. However, when it is desirable to express the velocity of hydrolysis in terms of the glucosidic linkages broken, it is necessary to calibrate the absorption ratios against the free aldehyde groups formed. Such calibrations were carried out in this work by removing two samples simultaneously at intervals from a hydrolysis mixture containing swine pancreatic amylase and waxy maize starch and determining the absorption ratio (AR) with one sample and the per cent theoretical glucose (*5, 14*) with the other. The per cent theoretical glucose was determined by an iodometric method (*5*) that had been modified to give greater sensitivity (*14*). Waxy maize starch concentrations of 0.05 and 0.025% and hydrolysis temperatures of 30° and 40° C. were used. The relationship between the absorption ratio and the per cent theoretical glucose was found to be linear within experimental error. It was not influenced by the changes studied either in the concentration of the substrate or in the temperature of hydrolysis. The best straight line by least squares gives $\frac{0.975 - AR}{0.076}$ = % theoretical glucose. This particular relationship is valid only for the hydrolysis of waxy maize starch by swine pancreatic amylase, which differs in its action from that of a number of other α-amylases (*7*). In addition, the accuracy of calibration curve used here is limited by any deviation of the iodometric method from stoichiometry.

Precision. The precision of the spectrophotometric method was established by determining the absorption ratios (AR) at 10-minute intervals for two identical hydrolysis mixtures containing

Table I. Determination of Absorption Ratios[a] for Duplicate Hydrolyzates[b] of Waxy Maize Starch and Crystalline Swine Pancreatic Amylase

Time of Hydrolysis, Minutes	Absorption Ratio[b]		Deviation from Mean, d[c] $d \times 10^3$
0	1.00	1.00	
10	0.932	0.945	6.5
20	0.803	0.786	8.5
30	0.689	0.675	7.0
40	0.572	0.591	9.5
50	0.500	0.493	3.5

[a] $AR = \frac{OD_t}{OD_0}$, where OD_t = absorbance of hydrolyzate at 540 mμ and at time t, OD_0 = absorbance of original waxy maize starch solution at 540 mμ.
[b] Waxy maize starch 0.00625%, 0.02M NaCl, 0.01M phosphate; pH 7.2.
[c] Standard deviation = ±0.007 AR unit.

0.0625 gram of defatted (*9*, *12*) waxy maize starch per liter. The data summarized in Table I show that the standard deviation for a single determination is ±0.007 AR units. From the relationship given above, this value would be equivalent to a standard deviation of ±0.09% theoretical glucose or $\pm 3.5 \times 10^{-7}$ mole of aldehyde groups per liter.

Limits of Concentration of Waxy Maize Starch. The lowest concentration of waxy maize starch that can be subjected conveniently to this technique is 0.00625%. It should be possible to study still lower concentrations by using a cuvette with a longer optical path. The upper limit of substrate concentration is fixed only by its solubility.

Inhibition Studies. Because iodine does not produce colored complexes with the lower molecular weight products of amylase action, the spectrophotometric method reported here can be adapted for a study of the inhibiting effects of these low molecular weight products on the initial velocity of the hydrolysis of branched components of starches by α-amylases.

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Activation Analysis of Trace Impurities in Germanium Using Scintillation Spectrometry

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A method has been developed, based on the use of neutron activation analysis, for the quantitative determination of trace impurities in germanium. Gamma scintillation spectrometry has been employed to identify and measure the amounts of the various impurities present. The method employs a minimum of chemical separation. A sensitivity of 0.001 to 1 γ is attained for most elements using this technique.

THE electrical properties of semiconductors, and the characteristics of devices in which they are used, are profoundly influenced by the presence of trace impurity atoms. In the case of germanium used in transistors, the concentrations of impurities are so minute that previously available methods of detection have proved ineffective.

Neutron activation analysis in conjunction with gamma scintillation spectrometry has been applied successfully to the analysis of trace impurities in silicon (*2*), and the present study concerns the development of a similar method for the analysis of trace impurities in germanium and some of its compounds. While methods using activation analysis have been developed in the past for the determination of traces of arsenic (*4*) and copper (*5*) in germanium, the present study takes advantage of the resolution of the gamma scintillation spectrometer, which permits the identification and measurement of many impurities in the same analysis with a minimum of chemical separation. The method possesses a sensitivity of 0.001 to 1γ for the majority of the elements.

NUCLEAR REACTIONS

When a sample is irradiated with thermal neutrons in a pile, many elements undergo an (n,γ) reaction with the formation of the corresponding radioisotopes. The amount of the radioisotope produced is proportional to the flux of neutrons involved, the reaction cross section, the weight of the element being investigated, and the duration of the irradiation. It is possible for other nuclear reactions such as (n,p) and (n,α) to occur in the case of a few elements, but it is accepted that the (n,γ) reaction will predominate when a sample is irradiated in a pile.

When germanium is irradiated, the (n,γ) reactions summarized in Table I occur. Three radioisotopes of germanium—germanium-71, germanium-75, and germanium-77—are formed, as well as radioactive arsenic-77—a product of the decay of germanium-77. Similarly, radioisotopes are formed from impurities present in the sample. Many of the nuclides formed from the impurities are gamma emitters. Consequently, gamma scintillation spectrometry is used to identify these impurities; it is based on the characteristic energies of the gamma photons of the respective nuclides.

Table I. (n,γ) Reactions of Germanium Isotopes

Naturally Occurring Stable Isotopes	Abundance, %	Isotopic Cross Section, Barn	Isotope Formed	Half Life	Method of Decay	Isotope Formed
Germanium-70	20.6	3.3	Germanium-71	11.4 days	K	Gallium-71 (stable)
Germanium-74	36.7	0.2	Germanium-75	45 sec.	β^-, γ	Arsenic-75 (stable)
		0.5		82 min.		
Germanium-76	7.7	0.015	Germanium-77	58 sec.	β^-, γ	Arsenic-77 (radioactive)
		0.30		12 hours		

Interference in the impurity analysis occurs as a result of secondary (n,γ) reactions during irradiation of germanium. Stable gallium and arsenic, formed by the decay of germanium, give rise to radioactive gallium-72 and arsenic-76, which are indistinguishable from gallium-72 and arsenic-76 formed from the corresponding impurity originally present in the sample. The reactions for their formation are:

$$Ge^{70} \xrightarrow{n,\gamma} Ge^{71} \xrightarrow{K} Ga^{71} \xrightarrow{n,\gamma} Ga^{72} \quad (1)$$

$$Ge^{74} \xrightarrow{n,\gamma} Ge^{75} \xrightarrow{\beta^-} As^{75} \xrightarrow{n,\gamma} As^{76} \quad (2)$$

It is possible to calculate the contribution of these secondary reactions and correct for their interference.

Another interference encountered in the spectrometric analysis of neutron-activated germanium samples is the presence of a number of large gamma photopeaks in the scan of the sample, due to the nuclides of germanium, the major component. These photopeaks interfere in the measurement of the photopeaks of the trace impurities. It is necessary, therefore, to separate chemically the radioactive germanium from the gamma-emitting impurities in the sample.

When germanium tetrachloride is irradiated, the chloride atoms form radioactive chlorine-36 and chlorine-38, with half lives of 4 $\times 10^6$ years and 37 minutes, respectively. Chlorine-36 is a pure beta emitter, while chlorine-38 decays by beta and gamma emission. Consequently these nuclides do not interfere in the measurement of activity because only the chlorine-38 emits gamma photons and measurement is made 8 hours after removal from the pile. Also, most of the radioactive chloride is removed during the chemical separation steps.

EXPERIMENTAL

Germanium Samples. The method has been developed to analyze for trace impurities in elemental germanium, germanium dioxide, and germanium tetrachloride, and may be applied with some modification to other germanium compounds.

Comparative Standards. Weighed amounts of pure elements or the appropriate compounds were irradiated with the samples for eventual comparison with the unknowns.

Germanium Standard Samples. There are no standard germanium samples available with known impurity content at the low concentration ranges concerned in this study; therefore, it was necessary to prepare synthetic standards to test the accuracy and precision of the method. Germanium and pure elements or compounds of the elements to be determined were irradiated simultaneously. The germanium had previously been analyzed by activation analysis and found to contain only 3.1×10^{-6} gram of arsenic per gram of germanium. The pure elements after irradiation were dissolved and small known aliquots were added to the radioactive germanium in a distilling flask and carried through the procedure.

Irradiation. A sample of appropriate size (0.1 to 1 gram of solid and 1 ml. of germanium tetrachloride), depending on the amount available and the anticipated purity of the material, together with the corresponding pure elements used as comparative standards, were sealed in separate quartz ampoules for irradiation in the Brookhaven pile. When irradiated for 3 days at a flux of approximately 3.4×10^{12} neutrons per square centimeter per second, most of the elements of the periodic table produce radioactive species. The maximum flux of the pile was employed to produce as high an activity as possible from the trace impurities for a 3-day irradiation; many of the isotopes approach saturation in this interval of time. The majority of these elements produce one or more radionuclides which emit gamma photons identifiable by the differences in the energies of these photons with the gamma scintillation spectrometer. Liquid germanium tetrachloride samples sealed in quartz ampoules were irradiated in the water-cooled facility of the pile at approximately room temperature.

Chemical Separations. Germanium, the major element, when irradiated under the above conditions, results in nuclides that emit gamma photons; therefore, it was necessary to separate chemically the radioactive germanium from the radioactive impurities so that the impurities could be measured with the gamma scintillation spectrometer. The distillation procedure employed was a modification of that used by Smales and Pate (*4*). Because of the relatively high activity of the irradiated germanium samples, some lead shielding of the distillation apparatus was necessary.

Elemental germanium samples after irradiation were crushed and dissolved with 50 ml. of aqua regia in a 100-ml. distilling flask. Irradiated germanium dioxide samples were dissolved in a distilling flask using 50 ml. of concentrated hydrochloric acid containing 1 ml. of concentrated nitric acid. Preparation of irradiated germanium tetrachloride samples for distillation was accomplished by adding it drop by drop to 10 ml. of water in a distilling flask and shaking until hydrolysis was complete. A mixture of 40 ml. of concentrated hydrochloric acid and 1 ml. of nitric acid was added to dissolve the germanium dioxide suspension, resulting in a single liquid phase that could readily be distilled.

Copper and arsenic carriers (25 mg. each) were added to the sample in the distilling flask. The copper served as a hold-back carrier for the nonvolatile impurities, whereas the arsenic carrier assisted in the subsequent distillation of arsenic trichloride. The solution was distilled down to a volume of 2 to 3 ml. under oxidizing conditions resulting from the addition of the nitric acid. This suppressed the distillation of arsenic. Addition of 10 ml. of mixed hydrochloric and nitric acids (9 to 1) and distillation to a small volume were repeated two more times. After the third distillation, the receiver containing the distilled germanium tetrachloride was removed and replaced by one containing 20 ml. of water. The delivery tube of the condenser was permitted to dip just below the surface of the water. Ten milliliters of 48% hydrobromic acid were added to the residue in the distilling flask to reduce arsenic(V) to arsenic(III), which was then distilled over as the trichloride. The solution was distilled to a volume of 2 to 3 ml. Addition of 10 ml. of hydrobromic acid and distillation were repeated twice more. The distillate in the receiving flask containing the arsenic activity was diluted to a known volume and an aliquot was taken for measurement of arsenic-76, using the spectrometer. The residue in the distilling flask containing the nonvolatile radioactive impurities was then transferred to a 50-ml. beaker, evaporated to dryness, and analyzed with the spectrometer.

In order to test the completeness of the separation of germanium from the impurities, the amount of germanium present in the arsenic distillate and the final residue was determined by measuring the germanium activity with the spectrometer. It was found that the arsenic distillate contained less than 0.1% of the germanium, whereas less than 0.01% of the germanium remained in the distilling flask. By greatly reducing the concentration of the gamma-emitting nuclides of germanium in the arsenic distillate and final residue, it was a relatively simple task to measure the arsenic and other gamma-emitting impurities with the spectrometer without any further chemical separation.

Measurement of Activity. All measurements of radioactivity were made with a gamma scintillation spectrometer, using a thallium-activated sodium iodide crystal as the detector. Details of the instrument and techniques employed are included in a previous paper by the authors (*2*). Further details on the use of scintillation spectrometry for quantitative measurement are described by Connally and Leboeuf (*1*). In all cases, appropriate decay corrections were applied in calculating the quantities of trace impurities present. Determination of the half life, along with the gamma energy of the photopeaks, established the radiochemical purity of the measured photopeaks.

Arsenic activity was determined by measuring an aliquot of the arsenic distillate with the spectrometer. Because gamma activity was measured, absorption effects by the liquid were negligible. The gamma photopeak due to arsenic-76 was measured and compared with the corresponding comparative standard after appropriate correction for radioactive decay. Arsenic-76 is also formed by a secondary nuclear reaction during irradiation of the germa-

nium sample, which necessitates calculating the amount produced (*3*, *4*) and subtracting this from the total arsenic-76 measured in the sample.

The residue containing the nonvolatile impurities was analyzed with the spectrometer; quantitative measurement of the respective impurities was accomplished by comparing the photopeaks with those obtained from the corresponding comparative standards. Because gallium-72 is formed by a secondary reaction during irradiation of germanium, the amount produced in this manner was calculated and subtracted from the total gallium-72 measured in the sample (*3*). It should be noted that with different conditions of time of irradiation and neutron flux, varying amounts of gallium and arsenic will result. Thus, it may be possible with the proper choice of conditions to minimize the effects of these reactions (*4*). The conditions of irradiation employed in this method, however, were chosen to activate as many trace impurities as possible in the same irradiation with consequent sacrifice of optimum conditions for minimizing these side reactions.

RESULTS AND DISCUSSION

Only those elements which result in radionuclides having half lives greater than approximately 4 hours and less than 200 days could easily be determined by this method. This interval was chosen as most convenient because measurement of the activity in the samples started approximately 8 hours after removal from the pile. Radionuclides with half lives much shorter than 4 hours could not be detected conveniently in this laboratory when present in trace amounts, whereas nuclides with half lives much greater than 200 days would be insufficiently activated for measurement under the conditions of the irradiation employed.

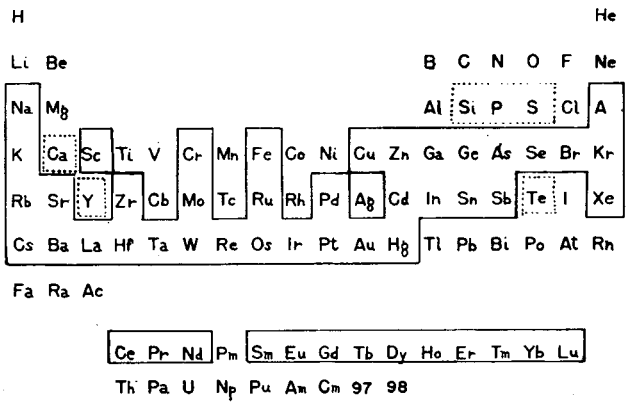

Figure 1. Trace elements producing measurable radioactivity

Based on a 3-day irradiation at a flux of 3.4×10^{12} neutrons per square centimeter per second and measurement of the short half-lived isotopes within 8 to 10 hours after removal from the pile. Elements in solid blocks form gamma-emitting radionuclides measurable on scintillation spectrometer. Elements in broken blocks form only beta-emitting radionuclides.

There are, of course, exceptions to this general classification, depending upon the nuclear properties of certain impurity elements. Thus, if the corresponding stable isotopes of these impurity elements possess optimum cross sections and abundances, the activity produced by irradiation may be sufficiently high for detection beyond the limits of the half lives mentioned. Also, if the impurities are present in larger amounts, greater activity will result. This method was designed to determine as many elements as possible with a single irradiation of a relatively pure sample of germanium; those elements which can conveniently be determined in trace amounts are shown in Figure 1.

The results of the analysis of several samples of germanium, germanium dioxide, and germanium tetrachloride are given in Table II. The arsenic values have been corrected for the amount of arsenic formed by the germanium during irradiation. Gallium was detected in all of the samples; however, the amount was equal to that which was calculated to be formed by the secondary nuclear reaction. Under the conditions of irradiation employed in this method, the apparent arsenic and gallium contents due to secondary nuclear reactions are 4.2×10^{-7} and 1.8×10^{-7} gram per gram of germanium, respectively.

Table II. Analysis of Trace Impurities in Germanium Samples

Sample	Impurity	Radionuclide	Measured Gamma Energy, M.E.V.	Measured Half life, Hours	Concn., %
Germanium dioxide	As	As^{76}	0.55, 1.22	26	1.5×10^{-4}
	Na	Na^{24}	1.37	15	3.9×10^{-5}
	Cu	Cu^{64}	0.51	12.5	1.6×10^{-6}
	Zn	Zn^{69}	0.44	14	1.4×10^{-3}
Germanium dioxide	As	As^{76}	0.55, 1.22	26	1.5×10^{-4}
	Na	Na^{24}	1.38	15	5.6×10^{-5}
	Cu	Cu^{64}	0.51	13	1.3×10^{-6}
	Zn	Zn^{69}	0.44	14	1.3×10^{-4}
Germanium dioxide	As	As^{76}	0.55, 1.22	26	2.2×10^{-4}
	Na	Na^{24}	1.38	15	2.4×10^{-5}
	Zn	Zn^{69}	0.44	14	2.2×10^{-4}
Germanium tetrachloride	As	As^{76}	0.55, 1.22	26	1.5×10^{-4}
	Na	Na^{24}	1.38	15	3.4×10^{-5}
	Cu	Cu^{64}	0.51	13	5.3×10^{-6}
Germanium tetrachloride	As	As^{76}	0.55, 1.22	26	3.8×10^{-4}
	Na	Na^{24}	1.38	15	2.5×10^{-5}
Germanium	As	As^{76}	0.55, 1.22	26	3.1×10^{-4}

Accuracy and Precision. The results of the analysis of the standard germanium samples are shown in Table III. The germanium used in the preparation of these samples had previously been analyzed and found to contain 3.1×10^{-6} gram of arsenic per gram of germanium. Consequently, the arsenic content of these samples is the sum of the arsenic originally present plus the known amounts added. The arsenic and gallium values obtained in the analyses have been corrected for the amounts of these elements produced in the secondary nuclear reactions during irradiation.

Table III. Analysis of Germanium Standard Samples

Germanium, Gram	Impurity Element	Amount of Impurity Element, Gram: Present	Amount of Impurity Element, Gram: Found	Error %
0.1538	As	1.3×10^{-6}	1.2×10^{-6}	− 7.7
	Ga	3.6×10^{-6}	3.7×10^{-6}	2.8
	Na	1.3×10^{-5}	0.95×10^{-5}	−27
0.2298	As	1.5×10^{-6}	1.3×10^{-6}	−13
	Ga	3.6×10^{-6}	3.1×10^{-6}	−14
	Na	1.3×10^{-5}	0.98×10^{-5}	−25
0.1366	As	1.2×10^{-6}	1.2×10^{-6}	0
	Ga	3.6×10^{-6}	2.9×10^{-6}	−19
	Na	1.3×10^{-5}	1.1×10^{-5}	−15
0.1476	As	1.1×10^{-6}	1.0×10^{-6}	− 9.1
	Na	6.6×10^{-5}	6.1×10^{-5}	− 7.6
	Zn	5.4×10^{-5}	3.9×10^{-5}	−28
	Cu	5.7×10^{-6}	3.9×10^{-6}	−32
0.1957	As	1.7×10^{-6}	1.7×10^{-6}	0
	Na	6.6×10^{-5}	4.8×10^{-5}	−27
	Zn	5.4×10^{-5}	3.8×10^{-5}	−30
	Cu	5.7×10^{-6}	3.9×10^{-6}	−32
0.1437	As	1.3×10^{-6}	1.2×10^{-6}	− 7.7
	Na	6.6×10^{-5}	5.9×10^{-5}	−11
	Zn	5.4×10^{-5}	3.8×10^{-5}	−30
	Cu	5.7×10^{-6}	4.0×10^{-6}	−30

Although the precision of the method is good, never exceeding an estimated standard deviation of 13% for triplicate analyses of the respective elements, the accuracy of the determinations appears to be low by as much as 30% in some cases. It has previously been shown that an accuracy and precision of about 1% can be obtained in the activation analysis of trace impurities in silicon and aluminum, where scintillation spectrometry is performed directly on the irradiated sample without any chemical

manipulation involved (*2*). The major part of the error in the present method, therefore, can be attributed to chemical loss during the separation procedure required before the gamma spectrometric analysis could be performed. The low values obtained in the determinations may be explained by the fact that it was impossible to remove all of the activity from the distilling flasks, even though quantitative washing techniques were employed. In every instance some of the activity from the residue adhered to the walls of the flask.

These determinations were performed on samples containing impurities in the range of 10^{-5} to 10^{-6} gram and involved chemical separations. In view of these conditions, the method exhibits good accuracy and precision.

ACKNOWLEDGMENT

Acknowledgment is gratefully given to D. H. Baird for his many valuable suggestions and criticisms.

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X-Ray Diffraction Patterns of Some Guanidine Derivatives

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X-ray diffraction patterns are a convenient means of characterizing crystalline compounds. Such data are presented here for a number of guanidine derivatives.

IN THE course of various studies, x-ray powder diffraction data have been accumulated on the following compounds: nitroguanyl hydrazone of acetaldehyde, phenylacetalnitroaminoguanidine, nitroguanyl hydrazone of cinnamaldehyde, benzalnitroaminoguanidine, nitroguanyl hydrazone of aceto-

Table I. X-Ray Diffraction Powder Data

d, A.	I/I_1
Nitroguanyl hydrazone of acetaldehyde	
12.27	1
8.94	3
7.97	1
7.25	9
4.71	5
4.45	10
4.08	6
3.56	5
3.40	9
3.20	7
3.04	4
2.85	7
2.68	5
2.56	2
2.43	5
2.35	1
2.27	1
2.15	2
2.04	1
1.93	2
1.82	1
1.77	2
1.72	1
1.63	1
1.58	1
1.53	1
1.50	1
Phenylacetalnitroaminoguanidine	
11.11	5
9.56	3
5.54	7
4.74	4
4.39	8
4.15	7
3.95	4
3.67	2
3.51	7
3.36	10
3.12	1
Phenylacetalnitroaminoguanidine (*Contd.*)	
2.98	2
2.84	1
2.71	3
2.64	1
2.35	2
2.26	1
2.13	2
1.97	2
1.65	1
1.52	1
Nitroguanyl hydrazone of cinnamaldehyde	
14.48	3
9.07	2
8.15	1
7.50	6
6.86	1
5.07	6
4.74	2
4.54	10
4.31	2
4.01	2
3.56	9
3.46	7
3.34	1
3.08	3
2.98	2
2.80	2
2.59	1
2.45	1
2.31	1
2.18	1
2.09	1
1.94	1
1.82	1
1.65	1
Benzalnitroaminoguanidine	
9.03	3
6.44	1
6.11	9
5.72	2
5.28	4
4.55	2
4.32	8
3.75	3
3.60	3
3.16	10
2.91	1
2.27	1
2.17	1
2.07	1
1.58	1
Nitroguanyl hydrazone of acetophenone	
13.91	4
12.54	10
9.31	1
8.63	1
7.02	1
6.54	6
5.77	1
5.47	7
4.77	4
4.56	4
4.30	6
4.02	1
3.83	4
3.60	4
3.31	6
3.17	1
3.05	5
2.97	1
2.81	1
2.72	1
2.54	2
2.38	1
2.33	1
2.16	1

Table I. (*Continued*)

d, A.	I/I_1
Nitroguanyl hydrazone of acetophenone (*Contd.*)	
2.09	1
2.02	1
1.96	1
1.87	1
1.81	1
Phenylnitroguanidine	
7.72	1
6.73	2
5.31	9
5.13	8
4.83	6
4.52	6
4.07	6
3.86	4
3.54	10
3.17	4
3.04	5
2.96	2
2.83	7
2.63	1
2.55	2
2.47	1
2.41	1
2.29	1
2.24	1
2.18	1
2.14	2
2.02	1
1.97	1
1.72	1
1.66	1
1-Acetamido-3-nitroguanidine	
7.17	8
5.50	2
5.08	1
4.34	10
4.09	9
3.76	2
3.52	5
3.12	2
2.98	7
2.86	2
2.74	5
2.56	1
2.47	5
2.37	2
2.32	2
2.25	1
2.18	2
2.12	2
2.03	1
1.97	2
1.94	1
1.89	2
1.85	1
1.75	1
1.66	1
1.59	1
1.54	1
1.39	1
1-Amino-1-methyl-2-nitroguanidine	
8.46	7
7.73	7
4.94	10
4.18	3
3.83	7
3.73	2
3.63	8
3.30	9
3.02	7
2.93	4
2.82	5
2.64	2
2.54	3
2.47	6
2.38	5
2.29	2
2.22	5
2.12	4
2.02	5
1.90	5
1.84	1
1.79	1
1.68	2
1.58	1
1.56	2
1.51	1
1.47	1
1.44	1
1.41	1
1.35	1
1.33	1
1.21	1
1.18	1
Methylnitroguanidine	
7.73	10
5.70	3
5.22	3
4.71	4
4.21	9
3.86	4
3.72	9
3.56	7
3.28	9
3.12	9
2.74	4
2.59	4
2.49	3
2.37	4
2.25	4
2.16	4
2.12	2
2.01	2
1.93	2
1.87	3
1.78	5
1.70	1
1.63	2
1.60	2
1.56	1
1.47	1
1.42	1
Nitroguanidine hydrobromide	
5.63	3
5.18	10
4.02	8
3.79	3
3.55	1
3.41	7
3.31	8
3.21	8
3.09	7
2.92	7
2.82	3
2.73	3
2.64	3
2.59	3
2.47	3
2.37	3
2.31	2
2.26	3
2.21	2
2.16	2
2.09	2
2.06	2
1.94	3
1.90	1
1.84	1
1.78	2
1.74	2
1.71	2
1.66	1
1.51	2
1.49	1
1.46	1
1.43	1
1.39	1
1.37	1
1.31	1
1.21	1
1.16	1
Nitroguanidine hydrochloride	
6.13	4
5.57	3
5.11	6
4.09	5
4.03	8
3.87	3
3.33	8
3.10	10
2.81	7
2.64	4
2.57	5
2.42	4
2.37	4
2.29	5
2.21	5
2.09	1
2.04	1
1.92	1
1.89	1
1.75	1
1.70	2
1.67	2
1.59	1

Table I. (*Continued*)

d A.	I/I_1
Nitroguanidine hydrochloride (*Contd.*)	
1.55	1
1.48	1
1.41	1
1.27	1
1.16	1
1-Benzylideneamino-1-methyl-2-nitroguanidine	
7.76	3
7.25	2
6.63	2
5.95	10
5.59	2
5.28	2
4.48	4
4.26	3
3.96	1
3.87	1
3.66	9
3.43	8
3.28	9
3.15	3
3.03	1
2.71	1
2.59	1
2.49	1
2.39	1
2.28	1
2.20	2
2.05	1
1.98	2
1.93	2
1.83	1
1.78	1
1.73	1
1.69	1
1.66	1
1.58	1
Nitroaminoguanidine	
7.90	1
5.72	5

d, A.	I/I_1
Nitroaminoguanidine (*Contd.*)	
5.23	1
4.77	4
4.41	7
3.93	7
3.40	7
3.22	10
3.05	4
2.91	6
2.66	4
2.57	1
2.47	3
2.34	3
2.28	2
2.21	5
2.13	4
2.05	1
1.95	1
1.88	5
1.73	4
1.64	3
1.61	3
1.56	1
1.33	3
1.30	1
Nitroguanidine	
7.40	1
6.44	1
5.66	1
5.17	9
4.52	2
4.22	7
3.64	5
3.30	8
3.08	10
2.91	8
2.64	7
2.52	3
2.42	5
2.34	3
2.26	1
2.18	2
2.13	2

d, A.	I/I_1
Nitroguanidine (*Contd.*)	
2.08	2
2.01	5
1.91	1
1.86	2
1.79	2
1.68	2
1.62	2
1.53	2
1.46	1
1.43	1
1.26	2
1.22	1
1.19	1
1.16	1
1.10	1
1.08	1
Benzalamino-guanidine	
15.50	5
8.19	3
5.52	5
4.89	4
4.37	10
4.10	7
3.73	1
3.62	1
3.49	1
3.34	6
3.19	4
3.09	9
2.93	1
2.79	1
2.64	1
2.59	5
2.25	3
2.04	1
2.00	1
1.91	1
1.83	1
1.77	1
1.62	2

phenone, *phenylnitroguanidine, 1-acetamido-3-nitroguanidine,* 1-amino-1-methyl-2-nitroguanidine, methylnitroguanidine, nitroguanidine hydrochloride, nitroguanidine hydrobromide, 1-benzylideneamino-1-methyl-2-nitroguanidine, nitroaminoguanidine, benzalaminoguanidine, and nitroguanidine.

Data on nitroguanidine have been given elsewhere (*1*), but are presented here because a number of differences are observed between the present data and those previously published.

EXPERIMENTAL

Samples were prepared by grinding small amounts in an agate mortar. They were then mounted in thin-walled glass capillaries of 0.3-mm. diameter. Film patterns were recorded in a 114.6-mm.-diameter camera, using CuK radiation filtered through nickel foil (λ = 1.5418 A.). The samples were rotated during exposure.

Interplanar spacings and the intensities for these compounds are given in Table I. Intensities were visually estimated and are reported on a basis of 10 to 1, where 10 represents the most intense line.

ACKNOWLEDGMENT

The materials for which data are given here were prepared by R. A. Henry, W. G. Finnegan, and Joseph Cohen.

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Determination of Hydrogen by Beta-Ray Absorption

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Despite the importance of the hydrogen content of liquid fuels, the petroleum industry seldom uses this property in process control because determining hydrogen by combustion is slow and complicated. An instrument has been developed that determines hydrogen in 5 to 20 minutes by measuring the β-ray absorption of a sample. Replicate determinations on six hydrocarbons show a standard deviation of 0.03% hydrogen, and single determinations have an average error of 0.03% hydrogen. Interference from elements heavier than carbon can be corrected by a simple factor.

MANY petroleum refining processes alter the hydrogen content of the feed. Catalytic reforming removes hydrogen from paraffins and cycloparaffins to produce aromatics. Solvent extraction selectively removes aromatic hydrocarbons from lubricating oils and leaves paraffins of higher hydrogen content. Even simple distillation may separate a feed into fractions of widely different hydrogen content. Thus, hydrogen content of organic liquids can be a useful index of process operation.

Because existing methods for determining hydrogen are slow, this property is seldom used. A skilled operator using conventional combustion techniques can analyze four to ten samples a day with an accuracy of 0.05 to 0.10% hydrogen. A method is needed to determine hydrogen rapidly with comparable accuracy by an unskilled operator.

An instrumental method that meets these requirements has been developed in this laboratory. The principle that hydrogen absorbs more β-rays than equal weights of other elements (*1*) underlies the operation of the instrument. Thus, a liquid absorbs more β-rays as its hydrogen content increases. This principle has also been used by Smith and Otvos (*3*).

The total energy loss, E, of β-rays in passing a distance, Z, through a liquid is approximately:

$$E = Z(L_H\rho_H + L_C\rho_C + \Sigma L_X\rho_X)$$

where L refers to the energy losses per gram per square cm. and ρ to the specific gravities for hydrogen, carbon, and other elements (X). The specific gravity of hydrogen ρ_H, is defined as

$$\rho_H = \rho \frac{\%H}{100}$$

where ρ is the density of the liquid and $\%H$ is weight per cent hydrogen. In the instrument, the energy loss in the liquid is proportional to

$$\rho_C + K_H\rho_H + \Sigma K_X\rho_X$$

where K_H and K_X are constants that relate the differences in energy loss per collision of β-rays with electrons of hydrogen and other elements to those with electrons of carbon. The expression

is evaluated by balancing the absorption in the liquid with that in a variable absorber and reading the value from a calibration chart prepared with known hydrocarbons. The instrument provides for simultaneous measurement of the specific gravity of the liquid. Per cent hydrogen is calculated from these two values.

Most hydrocarbon mixtures do not contain sufficient quantities of elements heavier than carbon to cause significant errors through contributions by the term $\Sigma K_X \rho_X$. If the amount of the other element is known, a simple correction can be applied.

APPARATUS

Essential features of the instrument are shown schematically in Figure 1. Two connected cells in a massive copper block, *G*, hold the liquid sample. The specific gravity of the liquid is determined by weighing a submerged plummet, *F*, suspended from the arm of a torsion balance by a platinum wire. The β-ray cell, *C*, is conical in shape. The axis of the cone passes through the centers of the source, *A*, and the ion chambers. Thin brass windows, so shaped that all β-rays traverse the same amount of the liquid, close the cell.

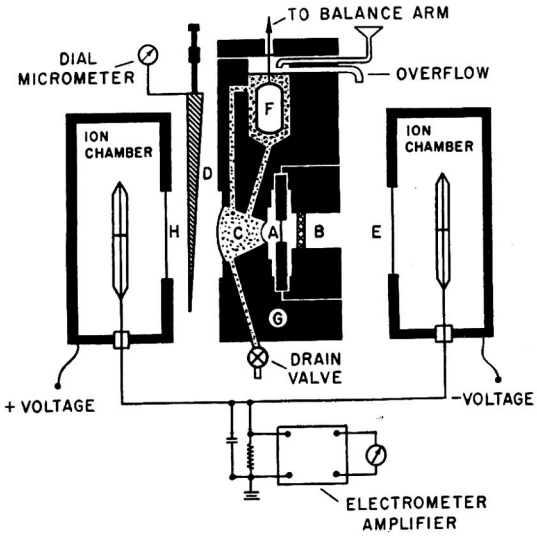

Figure 1. Schematic diagram of instrument

The copper block maintains the liquid in both cells at the same temperature. Calibration of the instrument thus does not depend on temperature. The block also serves as a radiation shield; less than 1 milliroentgen per hour can be detected outside the instrument.

The source of β-rays (*2*), a 10-mc. deposit of strontium-90 on a mica sheet, *A*, emits two equal beams in opposite directions. One beam passes through a constant Invar absorber, *B*, and through the thin window, *E*, into an ion chamber. The opposing beam passes through the sample cell, *C*, through a wedge-shaped absorber, *D*, and then through the thin window, *H*, into a second ion chamber. When the wedge is positioned so that the rate of absorption of β-rays in the sample plus the wedge equals the rate in the standard absorber, β-rays will enter both ion chambers at the same rate. Two shutters bracket the source and cut off the flow of β-rays to the ion chambers when the instrument is not in use.

Because opposite voltages are applied to the ion chambers, the currents oppose each other. An electrometer tube amplifies the net ion-chamber current, which is read on a galvanometer or recording potentiometer. When the galvanometer shows no deflection, β-rays are entering both ion chambers at the same rate. The wedge position corresponding to this condition is the "null wedge position" and is read on a dial micrometer.

Figure 2 is a photograph of a commercial model being manufactured under license.

CALIBRATION

Four calibrations are needed before the instrument can be used routinely. The volume of the specific gravity plummet must be determined. The constant K_H must be evaluated, and a calibration curve must be constructed by analyzing hydrocarbons of known composition. Finally, if liquids containing elements other than carbon and hydrogen are to be analyzed, correction factors for these elements must be determined.

The volume of the specific gravity plummet is calculated from its weight in air, its weight in a pure hydrocarbon such as benzene, and the density of the hydrocarbon.

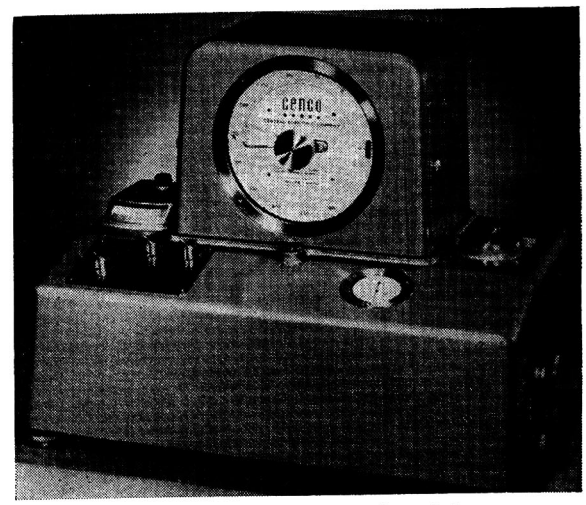

Figure 2. Commercial model

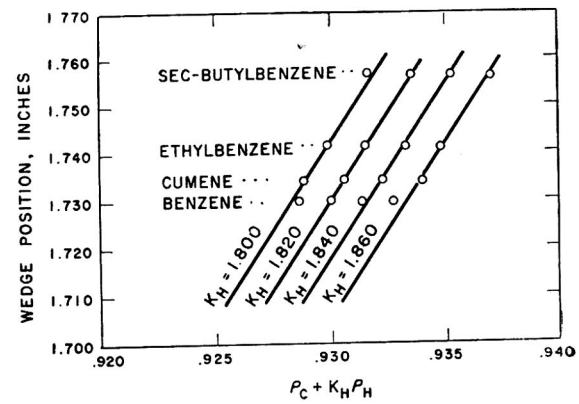

Figure 3. Evaluation of K_H

K_H is evaluated graphically; a simplified example is shown in Figure 3. Because K_H is critical for aromatics but less so for paraffins, four aromatic hydrocarbons, such as benzene, cumene, ethylbenzene, and *sec*-butylbenzene, are used. For each, the null wedge position is plotted against $(\rho_C + K_H \rho_H)$ for values of K_H in the neighborhood of 1.800. The plot must be large enough to indicate wedge position clearly to 0.001 inch and $(\rho_C + K_H \rho_H)$ to 0.0001 gram/ml. Attempts are made to draw smooth curves through the four points of constant K_H; that value of K_H, to 0.001 inch, that gives the smoothest curve is used in all subsequent determinations.

The calibration curve is constructed by determining specific gravity and null wedge position for a series of hydrocarbons, calculating $(\rho_C + K_H \rho_H)$ for each hydrocarbon, and plotting wedge position against $(\rho_C + K_H \rho_H)$. The scale of the plot should be the same as used in evaluating K_H. Phillips Pure Grade *n*-pentane, *n*-hexane, *n*-heptane, 2,2,4-trimethylpentane, cyclohexane, cyclopentane, benzene, toluene, ethylbenzene, cumene, and *sec*-butylbenzene are adequate for the calibration.

More accurate calibrations are possible for commercial instru-

Table I. Precision and Accuracy for Six Hydrocarbons

Hydrocarbon	% Hydrogen Calcd.	% Hydrogen Found[a]	Standard Deviation
n-Heptane	16.10	16.11	0.031
2,2,4-Trimethylpentane	15.88	15.90	0.035
Cyclohexane	14.37	14.40	0.032
Benzene	7.74	7.71	0.032
Cumene	10.06	10.06	0.015
Ethylbenzene	9.49	9.46	0.043

[a] Average of 11 determinations.

ments, which have wedges that taper linearly. The calibration points can be fitted directly to the equation

$$\rho_C + K_H\rho_H = aW + b$$

where a and b are constants and W is the null wedge position.

To determine correction factors for other elements, liquids containing these elements must be analyzed. Because most pure compounds fall beyond the range of the instrument, they must be diluted with aliphatic hydrocarbons. The specific gravity and null wedge position are determined for each mixture and used to calculate per cent hydrogen. The correction factor, F, for each element, X, is given by the equation

$$F_X = \frac{\%\text{H found} - \%\text{H true}}{\%X}$$

PROCEDURE

For each determination, the procedure consists of rinsing the sample cells, filling them with sample, measuring the specific gravity and the null wedge position, and draining the cells. Ten milliliters of liquid are sufficient to fill the cells. Two rinses with the sample are sufficient; no significant change in measured hydrogen content is noted between the third and fourth fillings. Viscous samples, such as lubricating oils, should be diluted with pure hydrocarbons. The null wedge position may be determined by either of two methods. Interpolation is more accurate, but the single-setting method is faster.

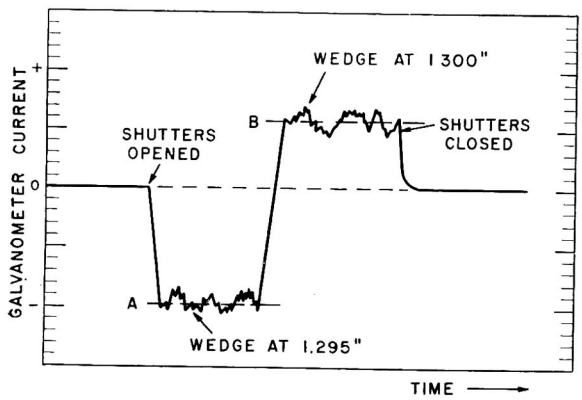

Figure 4. Determination of null wedge position

The interpolation method employs a recording potentiometer and is illustrated in Figure 4. The cells are rinsed and filled, and the mechanical and electrical zeros are adjusted. The wedge is brought to approximate balance, and the shutters are opened. The galvanometer current will vary about an average value. The wedge is then moved to swing the galvanometer across the zero point to a new average current. The null wedge position is calculated to within 0.001 inch by linear interpolation.

If a series of samples that give similar null wedge positions is to be analyzed, the procedure may be shortened. Because the ratio of change in galvanometer current to change in wedge position is constant over limited ranges, it can be determined for one sample and used with a single wedge setting for subsequent members of the series.

In the single-setting method, either the galvanometer or a recording potentiometer may be used. After the mechanical and electrical zeros are set, the shutters are opened. The wedge is then positioned so that the needle swings equally to the left and right of the zero. The null wedge position indicated on the dial micrometer is accurate within 0.002 inch.

While the wedge is being adjusted in either method, the specific gravity plummet is weighed periodically. The final weighing is made as the null wedge position is noted. For a complete determination, the first method requires 10 to 20 minutes; the second, about 5 minutes.

CALCULATION

Per cent hydrogen is calculated from the specific gravity of the sample and the null wedge position. The specific gravity, ρ, of the liquid is given by the equation:

$$= \frac{W_A - W_S}{V}$$

where W_A and W_S are the weights of the plummet in air and in the sample, respectively, and V is its volume. The value, N, of $(\rho_C + K_H\rho_H)$ corresponding to the null wedge position is read from the calibration chart. Hence:

$$\%\,H = 100\,\frac{N - \rho}{\rho(K_H - 1)} - \Sigma(F_X)\,(\%\,X)$$

An independent method must be used to determine % X.

Table II. Accuracy for Thirteen Binary Mixtures

	% Hydrogen Calcd.	% Hydrogen Found	Diff.
n-Hexane + *n*-heptane	16.23	16.15	−0.08
n-Heptane + toluene	11.97	11.97	0.00
n-Heptane + benzene	11.36	11.36	0.00
	13.58	13.51	−0.07
2,2,4-Trimethylpentane + benzene	11.32	11.32	0.00
2,2,4-Trimethylpentane + toluene	13.79	13.68	−0.11
2,2,4-Trimethylpentane + cyclohexane	14.65	14.67	+0.02
	15.24	15.27	+0.03
	15.57	15.59	+0.02
Cyclohexane + ethylbenzene	10.39	10.42	+0.03
	11.32	11.38	+0.06
	12.29	12.29	0.00
	13.31	13.31	0.00

RESULTS

Precision and accuracy of the instrument were studied by analyzing individual hydrocarbons and known and unknown mixtures. Reported results were obtained by the interpolation method on the pilot model of the instrument. A gradual increase

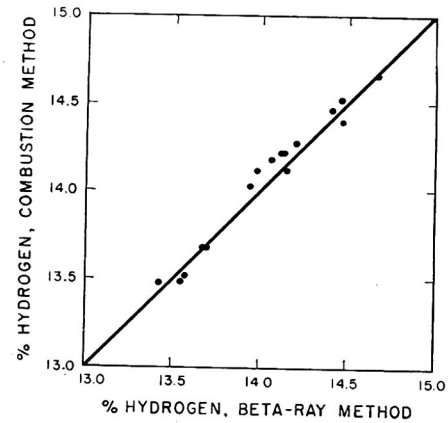

Figure 5. Comparison of β-ray and combustion methods

in the measured hydrogen contents corresponded to a uniform drift in calibration equivalent to 0.05% hydrogen per month. This drift was small enough that a daily check gave an adequate correction.

Six pure hydrocarbons were analyzed at weekly intervals. The data are summarized in Table I, where the average measured per cent hydrogen was calculated after correcting for drift. The average values do not differ from the calculated values by more than 0.03% hydrogen. The average standard deviation for all six hydrocarbons is 0.031% hydrogen; the average probable error is 0.021% hydrogen.

Analyses of thirteen known binary mixtures of hydrocarbons are given in Table II. The average difference between measured and calculated values is 0.032% hydrogen; the standard deviation of the differences is 0.050% hydrogen.

Three synthetic mixtures of hydrocarbons were also prepared and analyzed. Table III summarizes the composition and analysis of each mixture. The errors observed were no larger than than those found for individual hydrocarbons or binary mixtures.

Table III. Accuracy for Three Synthetic Mixtures

Composition of Mixture	Weight %		
2,2,4-Trimethylpentane	18.8	17.4	16.8
n-Heptane	18.4	17.2	6.5
n-Hexane	10.5	0.0	3.2
Cyclohexane	20.6	10.9	1.1
Methylcyclohexane	10.2	0.0	0.0
Methylcyclopentane	10.3	0.0	0.0
Benzene	0.9	1.8	2.3
Toluene	1.9	7.8	9.8
Ethylbenzene	3.7	18.0	23.6
Cumene	1.8	21.8	25.5
Butylbenzene	1.8	4.2	10.8
Isoprene	1.1	0.9	0.4
	100.0	100.0	100.0
Hydrogen			
Calculated	14.66	12.37	11.43
Found	14.67	12.34	11.40
Difference	+0.01	−0.03	−0.03

Table IV. Correction Factors for Six Elements

Compounds Used	% X	*F*	*K*[a]	Tolerance
Nitrogen				
Pyridine + iso-octane[c]	2.41	0.029		
Pyridine + iso-octane	4.62	0.025		
Av.		0.027	1.022	3.4
Oxygen				
Acetone	27.55	0.047		
Methanol	49.94	0.051		
2-Propanol	26.63	0.053		
1-Butanol	21.59	0.054		
Ethyl acetate + cyclohexane	4.09	0.066		
Ethyl acetate + cyclohexane	8.17	0.056		
Av.		0.054	1.044	1.7
Sulfur				
Thiophene + *n*-heptane	1.13	0.266		
Thiophene + *n*-heptane	5.37	0.245		
Thiophene + *n*-heptane	10.35	0.254		
Av.		0.255	1.208	0.4
Chlorine				
Chlorobenzene + iso-octane[c]	4.84	0.213		
Chlorobenzene + iso-octane	9.00	0.216		
n-Butyl chloride + cyclohexane	4.38	0.237		
n-Butyl chloride + iso-octane	9.30	0.219		
Chloroform + *n*-heptane	17.15	0.220		
Av.		0.221	1.181	0.4
Bromine				
Bromobenzene + *n*-heptane	10.78	0.569		
n-Butyl bromide + iso-octane[c]	10.18	0.563		
Av.		0.566	1.464	0.2
Lead				
Tetraethyllead + iso-octane[c]	0.95	1.593		
Tetraethyllead + iso-octane	5.26	1.609		
Tetraethyllead + iso-octane	7.00	1.641		
Av.		1.614	2.323	0.06

[a] *K* for carbon arbitrarily = 1.000; *K* for hydrogen = 1.820.
[b] Per cent that causes an error 3 times the standard deviation.
[c] 2,2,4-Trimethylpentane.

Seventeen liquid fuels were analyzed by both the β-ray instrument and conventional microcombustion. The data are plotted in Figure 5. β-Ray values represent single determinations. Combustion values are averages of two determinations on each sample; the average standard deviation for the duplicate values is 0.055% hydrogen. The average difference between the two methods is 0.054% hydrogen, and the standard deviation of the differences is 0.066% hydrogen. Student's *t* test shows that neither method gives significantly higher or lower results.

To determine the magnitude of errors caused by elements other than carbon and hydrogen, liquids containing six elements that occur in petroleum samples were analyzed. Table IV summarizes the composition of the mixtures and indicates by the empirical correction factors, *F*, the error in per cent hydrogen introduced by each per cent of the other element. The theoretical *K* constants are derived from the correction factors by the equation

$$K_X = 1 + F_X (K_H - 1)$$

The tolerance is taken as the per cent of each element that will cause an error three times the standard deviation, or 0.093% hydrogen. Sulfur and lead are the only elements commonly found in petroleum stocks in large enough amounts to require corrections.

Precision and accuracy of the single-setting method with a galvanometer depend on the skill of the operator in visually estimating the average position of the galvanometer needle. Limited experience of a few operators suggests a standard deviation of about 0.05% hydrogen.

DISCUSSION

The instrument meets the analyst's requirements of speed, accuracy, and precision. A standard deviation of 0.03% hydrogen represents a precision greater than that possible by conventional combustion techniques. The analyses of mixtures show that the accuracy of the determination is also satisfactory. Operation of the instrument is simple; a sample may be analyzed in less than 20 minutes—a decided advantage over the combustion method. Because commercial instruments have larger β-ray sources than the pilot model, they have greater precision.

Although the half life of the β-ray source is 25 years, the decrease in intensity is about 0.1% per week. Because the instrument employs two opposing beams of β-rays, changes in intensity do not affect the calibration. However, the statistical uncertainty in measured hydrogen content increases as the source ages. The estimated replacement time is 25 years.

Corrections for elements other than carbon and hydrogen are the only factors that may limit the accuracy of the instrument. Satisfactory performance with hydrocarbons points to many possible applications in the petroleum industry. Applications to hydrocarbons in other fields have not been explored, but the instrument should find many further uses.

ACKNOWLEDGMENT

The authors acknowledge contributions to the development of the instrument by Evon C. Greanias, and are indebted to P. K. Winter of the General Motors Research Laboratories for the chemical analyses of the liquid fuels.

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Flame Photometric Determination of Manganese in Cement

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A method for the flame photometric determination of strontium has been extended to the determination of manganese in portland cement and in portland blast-furnace slag cement. The instrument used is the Beckman DU flame photometer with oxyhydrogen flame and the photomultiplier attachment. No prior chemical separations are performed and the procedure is such that sodium, potassium, strontium, and manganese can be determined flame photometrically on the same sample solution.

THE determination of manganese is often included in a chemical analysis of cement. Portland blast-furnace slag cement may contain up to 1.5% manganese trioxide (*2*). Portland cements, on the other hand, rarely contain more than a few tenths of a per cent of manganese as the trioxide.

The manganese in cement is usually determined by the bismuthate or permanganate methods as described in the American Society for Testing Materials (*1*) and federal specification (*8*) standards for cement analysis. In the permanganate method the manganese is titrated with standard potassium permanganate solution. The bismuthate method involves oxidation of the manganese to permanganate with sodium bismuthate, and titration of permanganate with standard sodium arsenite solution.

The flame photometer is now firmly established as a standard tool for the determination of the alkalies and the alkaline earths. Several investigators have also reported its use for the determination of manganese in various materials (*7, 9–11*). Because methods are now available for the flame photometric determination of sodium, potassium, and strontium in a single 1-gram sample of cement (*5, 6*), it was decided to investigate the possibility of determining the manganese in this same sample by the use of a similar procedure.

This study was made using a Beckman DU spectrophotometer with model 9200 flame photometer, oxyhydrogen burner, and photomultiplier attachment.

EXPERIMENTAL

It was known that potassium emitted at a wave length very near that of manganese and might interfere seriously with its determination (*4*). Preliminary experiments with pure salts showed that a very sharp peak in the manganese emission occurred at about 403.3 mμ. Potassium was found to give a similar sharp peak of much lower intensity at about 404.5 mμ. For metal oxide concentrations up to 100 p.p.m., these peaks were readily resolved by the flame photometer, which has a half-intensity band width of 0.2 mμ at the slit width and wave length used (*3*). There was a slight residual interference of potassium with the manganese emission, but this was judged to be insignificant for this analysis.

It was necessary to make a correction for background emission due to the other constituents of cement. It was established that this could best be made by determining the emission at about 401.0 mμ, and subtracting it from the emission at 403.3 mμ. Because the primary purpose of this study was to see if a procedure similar to those already in use for the determination of sodium, potassium, and strontium could be used for manganese, this was tried; no detailed investigation of the exact interferences involved was made.

The following procedure was used. One-gram samples of cement were suspended in water, dissolved in 5 ml. of concentrated hydrochloric acid, diluted with water, digested, filtered to remove the small insoluble residue, and diluted to 100 ml. in volumetric flasks. These solutions were then compared with a series of standards made up from a low-manganese cement of previously determined manganese content. A standard manganese solution containing 100 p.p.m. of manganese trioxide was prepared by dissolving 0.3481 gram of electrolytic manganese metal in a little hydrochloric acid and diluting to 1 liter. The metal was examined spectroscopically and determined to be better than 99.9% pure. One-gram samples of the low-manganese cement were treated in the same manner as the sample, and the required volumes of standard manganese solution were added to each to give standards containing 10, 20, 40, 60, 80, and 100 p.p.m. of manganese trioxide.

The comparisons were made by setting the $\%T$ dial at 100, atomizing the 100-p.p.m. manganese trioxide standard, and balancing the galvanometer by means of the sensitivity control on the monochromator at a wave length of 403.3 mμ. A cement solution to be analyzed was then atomized, the meter balanced by the $\%T$ control, and the per cent transmittance recorded. The wave length was then set at 401.0 mμ, the meter again balanced by means of the $\%T$ dial, and the value recorded. After a short rinse with water, the process was repeated one or more times and the readings were averaged. This was then done for the other cements and reference standards. The details of manipulation and other instructions are given in the manufacturers' manuals (*3, 4*). The instrument was set about 1 to 2 turns from the counterclockwise limit of the sensitivity control on the monochromator, with a slit width of 0.02 mm., zero suppression control off, selector switch at .1, and full sensitivity on the photomultiplier battery box control. The manganese line at 403.3 mμ was found to be so sharp that it was not possible to reset the peak by merely resetting the dial. It was necessary to reset by slowly varying the wave length until maximum signal was obtained, as observed by the swing of the galvanometer needle.

Table I. Precision and Accuracy of Results

Sample	Mn_2O_3, %				
	Colorimetric	Flame Photometric			
		SiO_2 present		SiO_2 absent	
Portland cement	0.049	0.048	0.050	0.049	0.045
	0.26	0.25	0.25	0.26	0.26
Portland blast-furnace slag cement	0.60	0.58	0.60	0.60	0.60
White portland cement (reference standard)	0.013				

RESULTS

To obtain some data on the precision and accuracy of the recommended method of analysis, the manganese was determined in two portland cements and a portland blast-furnace slag cement. The results are shown in Table I. Each of the duplicate flame photometric values was obtained on a separate sample on different days.

These cements were also analyzed colorimetrically as a check on the flame photometric method. This same colorimetric procedure was used to determine accurately the manganese trioxide content of a white portland cement, which was then used as the low-manganese reference cement in the flame photometric procedure. The colorimetric results shown are the averages of two independent determinations made on different days. The determinations were made on samples with silica present, and from which the silica had been eliminated by a single dehydration with nitric acid. Results were obtained by oxidizing the manganese to permanganate with potassium

periodate in the presence of nitric and phosphoric acids, and measuring permanganate intensities spectrophotometrically.

The data in Table I indicate that the precision and accuracy are satisfactory. Flame photometric determinations made on samples from which silica had or had not been removed by a single dehydration with hydrochloric acid were equally satisfactory when compared with standards similarly treated.

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Determination of Water in 1,1-Dimethylhydrazine and Hydrazine by a High Frequency Method

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The systems water–1,1-dimethylhydrazine and water-hydrazine were studied using a Sargent Model V oscillometer. The data obtained for the two hydrazine systems are presented as plots for the range from 0 to 100% water. With the procedure used in this work the results for the range from 0 to 5% water in 1,1-dimethylhydrazine indicated an accuracy of 20 parts per thousand. However, in the range from 0 to 10% water in hydrazine the variation of results was too large for analytical application.

HYDRAZINE or 1,1-dimethylhydrazine from commercial sources may contain as much as 4 to 7% water. There is, at present, no simple or direct method for the determination of the water content. From the analysis of the hydrazine content by the direct iodate method, water may be obtained by difference (*5*); however, dissolved salts and ammonia (*1–3*) may interfere. A direct method of analysis is desirable.

The analysis of binary mixtures of water and organic compounds by means of high frequency methods has been reported (*9, 10*). The results obtained led this laboratory to investigate the applicability of this method to the analysis of binary mixtures of water and hydrazine or 1,1-dimethylhydrazine.

The theory of high frequency analysis (*7, 8*) indicates that the analysis of one component of a binary mixture is possible if the two liquids have sufficiently different dielectric constants. The dielectric constant of water is 80 and that of hydrazine is 55; therefore, analysis of water in hydrazine was thought to be possible by high frequency methods. An estimate of the dielectric constant of dimethylhydrazine by this laboratory was less than 10. Therefore, even better possibilities for success exist in this binary system than in the hydrazine-water system.

APPARATUS

A Sargent Model V oscillometer was used throughout this study with a small cell of the test tube type and an appropriate cell holder. To improve stability of operation, the Center Adjust condenser was mounted firmly on a rigid bracket and the ground lugs attached to each fixed range switch condenser were soldered to the common ground wire that runs parallel to the lugs. The instrument then proved to be stable following a 1.5-hour warm up. Drift was less than 5 range dial units per hour and minor mechanical vibration had no adverse effects.

This laboratory found, as have others (*7, 10*), that the position of the cell in the holder influences the reading obtained. This was overcome by inserting a banana plug into the bottom of the cell and a banana jack into the cell holder. These modifications ensured constant uniformity of the position of the cell in the holder and also eliminated wear of the plating on the inner electrode. The position of the cell holder, relative to the instrument, was fixed by mounting the holder on a large wooden platform. The length of the coaxial cable connecting the cell holder and the instrument was 6.5 inches.

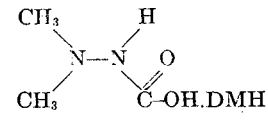
The cells, equipped with Teflon stoppers, excluded atmospheric oxygen and moisture satisfactorily. The dial units obtained as a result of measurement depend upon the temperature. To avoid the effects of temperature drift, the sample cell should be held at the top only, and the temperature of the room should be reasonably constant. Checks of room temperature in this laboratory indicated a total change from morning to night of about 1° C. The temperature coefficient of water was found to be about −30 range dial units per ° C., which agrees with data in the literature (*7, 10*). Between experiments the cell was cleaned with distilled water, then dried with acetone and jet of nitrogen gas through a glass tube drawn sufficiently small to pass between the walls of the cell. Care must be taken, when cleaning the cell, not to wet the external surface (*7*). The cell was filled by means of a pipet which was modified to deliver about 8.5 ml., thus filling the cell to 0.5 inch above the plated electrodes. One-ounce polyethylene bottles were used as sample containers for the hydrazine solutions.

PURIFICATION OF HYDRAZINE AND DIMETHYLHYDRAZINE

The hydrazine was obtained from Fairmount Chemical Co., Newark, N. J., and assayed 95%. After purification by vacuum distillation from fused potassium hydroxide (*1*) the assay was 99.8% hydrazine (*5*). The distillate was stored in a refrigerator in a borosilicate glass flask. The ground-glass stopper of the flask was sealed with "para gum-rubber" (Central Scientific Co.) tape and with clear plastic spray over the surface of the tape.

The 1,1-dimethylhydrazine was obtained from Aerojet-General Corp., Azusa, Calif.

1,1-Dimethylhydrazonium 2,2-dimethylcarbazate



was identified as being present in some lots of the dimethylhydrazine. This compound sublimes at about 57° C., collects in the condensing head of the column during atmospheric distillation of the dimethylhydrazine, and is slowly washed into the receiver by the condensate.

To eliminate this contamination, the top of the column was fitted with a three-way stopcock having a bore opening of 0.5

inch. To one arm of the stopcock a reflux cold finger was attached; to the other, the fractionating take-off head. Before fractionation the still was operated under total reflux. The vapors condensed on the cold finger where the sublimate collected. After refluxing 1 hour, the sublimate had been removed; the stopcock was turned to the vapor dividing head (Shell design) and the fractionation was started. Until the temperature plateau was reached, the ratio used was 1 to 20. This was advanced to 1 to 10 while the distillate was collected. The fraction collected over a 4-hour interval boiled at 60.63° to 60.75° C. at a barometric pressure of 699 to 700 mm. of mercury. A 4-foot vacuum-jacketed column, packed with $^1/_8$-inch glass helices, was used for this distillation. A nitrogen atmosphere was used to flush out the column before and during the distillation. The tank nitrogen was dried with sodium hydroxide and Drierite. Oxygen was removed by two Oxsorbent washing towers and by a copper turnings scavenger tube heated to 450° C. in an electrical furnace. The purified dimethylhydrazine assayed 99.8%.

EXPERIMENTAL

The following procedure was used in handling the purified materials for all preliminary studies and in establishing the relationship of dial unit readings with per cent of water in the samples. Working in a dry box is inconvenient, so a method was devised, by use of an intermediate container, for transferring the purified materials to separate polyethylene sample bottles.

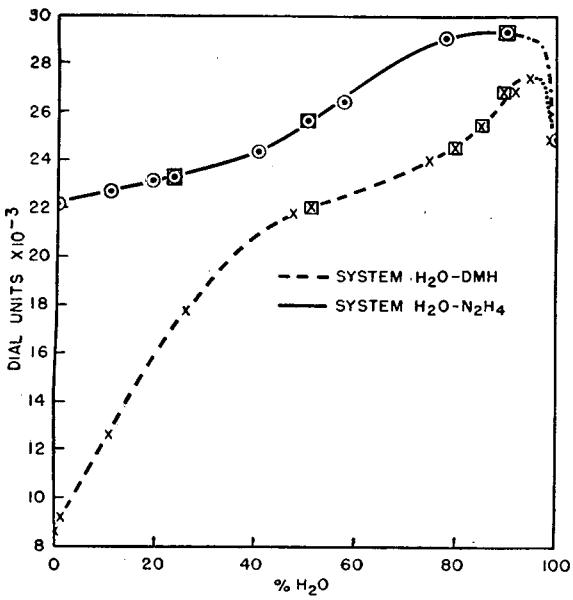


Figure 1. Water-hydrazine and water-dimethylhydrazine systems

Points enclosed in squares were obtained from solutions prepared 1 month later

The intermediate container consisted of a 3-necked, 300-ml. round-bottomed flask. The center neck was covered with a thin rubber membrane held in place with rubber bands. A small hole, approximately $^3/_{16}$ inch in diameter, was punctured in the diaphragm. One side neck served as a nitrogen gas inlet. The other side neck was stoppered after introducing through it a sufficient quantity of the hydrazine for the number of samples desired.

The polyethylene bottles were cleaned, dried, weighed, and filled with nitrogen prior to making up the water-hydrazine solutions. A 10-ml. pipet was inserted through the hole in the diaphragm and filled by variation of the pressure of nitrogen in the transfer flask. The cap was removed from a polyethylene bottle and the hydrazine delivered to the bottle. Reweighing of the bottles filled in this manner gave the weights of the hydrazine. Known weights of water were then added to prepare any series of solutions. The solutions were well mixed by shaking and allowed to stand for 1 hour to come to room temperature prior to making oscillometer measurements.

The empty cell holder was used for the zero reference when making the oscillometer measurements. The cell was cleaned, dried, and filled with nitrogen prior to addition of 8.5 ml. of solution with the special pipet.

All work with the hydrazines was performed in a hood, and rubber gloves were worn by the operator. The hydrazines may cause serious physiological effects; therefore, vapors from these materials should not be inhaled nor should the liquids be allowed to come into contact with the skin (*6*).

DATA

Data for the water-hydrazine and water-dimethylhydrazine systems are shown in Table I and plotted in Figure 1. Dial units represents the sum of the units of range switches and the range dial required to restore null deflection of the oscillometer after introduction of the sample.

Table I. Water-Hydrazine and Water-Dimethylhydrazine Systems

Water-Hydrazine		Water-Dimethylhydrazine	
Water, %	Dial units	Water, %	Dial units
0	22,200	1.00	9,250
10.8	22,700	10.5	12,630
19.6	23,180	26.5	17,650
23.5[a]	23,290	47.8	21,780
40.4	24,370	51.2[a]	22,130
50.3[a]	25,680	74.7	24,010
57.4	26,410	79.8[a]	24,580
78.0	29,090	85.1[a]	25,410
89.5[a]	29,430	90.0[a]	26,790
90.2	29,340	91.2	26,570
100	24.870	94.7[a]	27,390
100[a]	24,820	100	24,820
		100[a]	24,850

[a] Data were obtained from solutions prepared 1 month later and are plotted with a square enclosing the point.

The data for the calibration curve of water in dimethylhydrazine are given in Table II. A plot of these data reveals a linear relationship.

The water content of interest to this laboratory was in the range of 0 to 5%. Solutions containing water within this range were prepared and the statistical data obtained are given in Table III, *A* and *B*. The values for per cent water reported in Table III, *A* were obtained by interpolation from the calibration curve data of Table II. The values for per cent water reported in Table III, *B* were obtained by interpolation between the values for pure dimethylhydrazine corrected for water content and a standard containing 0.931% water, assuming a linear relationship.

Table II. Water-Dimethylhydrazine Calibration Data

Water, %	Dial Units	Water, %	Dial Units
0	8,840	5.87	11,040
1.00	9,250	6.86	11,380
1.94	9,610	6.95	11,400
2.85	9,900	7.59	11,630
3.03	10,020	10.2	12,550

The indexes of precision used in the statistical analysis of data presented in Table III are as follows:

Standard deviation (estimate) $s = \sqrt{\Sigma(x - \bar{x})^2/n - 1}$
Standard deviation of mean of n $S_m = s/\sqrt{n}$
95% confidence range (fiducial limits) $= \bar{x} \pm ts_m$

In the above

$\bar{x}$ = mean of n observation of x
t = Student's t for the significance level desired and $n - 1$ degrees of freedom (*4*). For the 95% significance level and means of 7 (6 degrees of freedom) $t = 2.45$

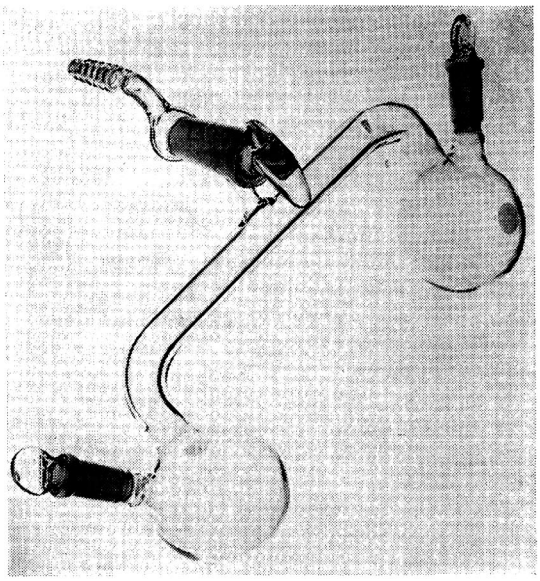


Figure 2. Vacuum distillation unit

The effect of dimethylamine upon dimethylhydrazine readings was desired because of evidence showing the presence of dimethylamine in commercial dimethylhydrazine. A stock solution of 10% by weight of dimethylamine in dimethylhydrazine was prepared. From this stock solution, standard curve solutions of dimethylamine in dimethylhydrazine were prepared by dilution with dimethylhydrazine following the same procedure used in the preparation of the water-dimethylhydrazine standard curve solutions. The data for the variation of dial units of dimethylhydrazine with respect to the dimethylamine concentration are shown in Table IV.

DISCUSSION

The data show that analysis of the binary system, water-dimethylhydrazine, can be performed satisfactorily by this high frequency method. Interfering substances in the analysis of commercial lots of dimethylhydrazine may be dimethylamine and the salt 1,1-dimethylhydrazonium 2,2-dimethylcarbazate. A correction can be made for the presence of dimethylamine if its concentration remains fairly constant. The salt was found only in a mixture of two lots of dimethylhydrazine; it probably would not be encountered as a frequent contaminant. The presence of a small percentage of this salt gives an apparent large increase in water concentration. If the salt is present, it may be removed without changing the water-dimethylhydrazine-dimethylamine ratio by a closed system total reflux arrangement similar to that described for the distillation of the dimethylhydrazine.

The analysis for dimethylamine in water-free dimethylhydrazine solutions may be performed by high frequency methods. The sensitivity of such an analysis might be improved if a cell compensator (7) were used. The data in Table IV indicate that the slope of the curve dimethylamine-dimethylhydrazine is opposed to that of the water-dimethylhydrazine curve. Comparison of these data indicates that at the 1% level of concentration the change in dial units is −20 and +360, respectively.

Table III. Determination of Water at Two Levels in Dimethylhydrazine

A. Approximately 2%

% Water Present	% Water Found	% Recovery
2.36	2.36	100.0
2.15	2.21	102.8
2.06	2.08	101.0
2.23	2.27	101.8
2.07	2.08	100.5
1.94	1.90	97.9
1.84	1.82	98.9

Average $\bar{x}$ = 100.4%
Standard deviation, s = 1.67%
Standard deviation of mean (of 7) s_m = 0.63%
95% confidence range = 100.4 ± 1.54%

B. Approximately 0.70%

% Water Present	% Water Found	% Recovery
0.74	0.71	95.9
0.74	0.74	100.0
0.72	0.72	100.0
0.70	0.70	100.0
0.69	0.68	98.6

Average $\bar{x}$ = 98.9%
Standard deviation, s = 1.78%
Standard deviation of mean (of 5) s_m = 0.80%
95% confidence range = 98.9 ± 2.21%

Table IV. Effect of Dimethylamine in Dimethylhydrazine

DMA, %	Dial Units
0	8430[a]
1.31	8410
2.61	8370
3.10	8350
10.0	3310

[a] Zero reading for 100% dimethylhydrazine different from that shown in Table II because of cell change.

Analysis of the water-hydrazine system should be possible by high frequency methods. It was found in this laboratory, however, that with the procedure used the precision of the analysis was below an acceptable limit. The variation of results was found to increase with decreasing water concentration, becoming too large for analytical purposes at concentrations as low as 0 to 10% water.

A possible contributing factor to the nonreproducibility of results with small amounts of water in hydrazine may have been the use of a glass storage container for the pure stock hydrazine. It has been found that the use of polyethylene instead of glass as sample containers increases the precision of the method; however, hydrazine stored in glass showed a dielectric constant of 55. The pure dimethylhydrazine was also stored in glass, but apparently this had no effect upon the reproducibility of results with this material. The oxidation of hydrazine or the absorption of water by the hydrazine are sources of error that may not have been entirely eliminated by the described procedure.

The analysis of commercial hydrazine is subject to the errors just mentioned, as well as the effect of dissolved solids. An apparatus, Figure 2, was made in this laboratory to remove these solids by vacuum distillation in a closed system. The commercial hydrazine was placed in one bulb and frozen in a dry ice–acetone bath. The pressure was then reduced to less than 1 mm. of mercury, the vacuum stopcock was closed, and the other bulb placed in a dry ice–acetone bath. The bulb containing hydrazine was then heated. A closed system distillation of the hydrazine was thus carried out.

The dial units for commercial hydrazine were found to be 26,890, while those of commercial hydrazine distilled as described above were found to be 22,550. By interpolation from Figure 1, presence of about 7% water is shown. The commercial hydrazine assayed 95% base. Therefore, dissolved material may be removed from the hydrazine by the above method prior to oscillometric analysis without changing the water to hydrazine ratio.

The data indicate that with the method described an analysis for small amounts of water in hydrazine is not feasible. Analysis of water in commercial 1,1-dimethylhydrazine, if corrected for the

presence of dimethylamine, would be expected to be accurate to within 20 parts per thousand.

ACKNOWLEDGMENT

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This paper has been released for publication by W. B. McLean, Technical Director of the Naval Ordnance Test Station.

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Total Porosity and Particle Density of Fluid Catalysts by Liquid Titration

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The phenomenon of loss of flow properties of a fluid catalyst when a liquid is added has been applied to measurement of total pore volume, which is equal to the liquid volume to reach a caking end point less that for a nonporous powder of the same size. The test has a standard deviation of less than 0.01 cc. per gram, can be carried out in 15 minutes, and requires very simple equipment. Results on fresh cracking catalysts are in excellent agreement with values obtained by the conventional nitrogen desorption method, but those for hydroformer and equilibrium cracking catalysts are slightly higher. The difference is believed to be a measure of macroporosity. By continuing the titration to a fixed volume, particle density and skeletal density can also be determined.

POROSITY is one of the most important properties of adsorbents and catalysts because diffusion to and from the internal surface is often a rate-determining factor in their performance. Surface area and, hence, activity stability, may be related to pore wall separation (*2*), the average value of which can be calculated from the pore volume–surface area ratio. Particle size measurements based on Stoke's law require knowledge of particle density, which is usually calculated from porosity and skeletal density (*5*).

Pore volume is commonly determined by sorption or desorption measurements near saturation pressures (*3*, *6*). Because of the necessity for very careful temperature control and long equilibration time, it is impractical to use pressures over about 97% of saturation, so that these measurements (*4*) according to the Kelvin equation for capillary condensation, commonly measure only microporosity (pores with a pore diameter under about 800 A.).

Because there was doubt as to whether data obtained by nitrogen desorption gave a true measure of the total porosity of cracking catalysts—i.e., whether macropores were present—and because a more rapid method was needed, a titration method for total porosity was developed. Macroporosity, which is the difference between total porosity and microporosity, was also of interest; equipment for measuring this by mercury penetration (*7*) was unavailable.

Table I. Pore Volume with Different Liquids

Liquid	Pore Volume Cc./Gram
Water	0.54
Benzene	0.55
Isopropyl alcohol	0.56
Acetone	0.65

PRINCIPLE OF METHOD

When a liquid is added to an agitated porous powder, the powder usually appears dry and free flowing up to a point, after which further small addition of liquid brings about a marked change in its properties. This behavior is readily explained by the fact that the liquid first fills in the pore space, after which the addition of a very small increment effects the same drastic change in properties as observed with nonporous powders. That is, the particles stick together to form a coherent caked mass. This behavior can be attributed to a very thin layer of liquid on the outside of the particles, which makes them stick together because of reduction of liquid-air interfacial surface energy. These phenomena were adapted to pore volume measurement.

Selection of Liquid. In principle, any liquid that wets the solid and is not too volatile or too viscous to penetrate the pores in a reasonable period of time could be employed. Because the forces which cause caking are due to the surface tension of the liquid, a material with high surface tension would be expected to give a sharper end point. Water appears to be an ideal material for porous inorganic materials that do not undergo swelling on contact with water. Clay cracking catalyst exemplifies a material for which the method may not be suitable because of swelling.

To test the hypothesis that other liquids might be used, several were tried on uncalcined cracking catalyst using the procedure detailed below. The limited results obtained are in Table I.

Reasonable agreement was obtained except for acetone, which is, of course, too volatile for this usage.

End Point Criteria. Evaluation of several end-point criteria led to the conclusion that caking with consequent adherence of powder to sides and bottom of the containing vessel was a sharp, reproducible end point which could be reversed by addition

of more sample. The size of the sample and vessel was not found to be critical within normal limits. Vessel dimensions are of some importance, however, because the tendency to adhere to the sides and bottom is in some degree a function of height and diameter occupied by the sample. (The tendency to flow on upending decreases with an increase in length-diameter ratio.) Data obtained on the same material with different sample sizes and bottles are given in Table II.

Table II. Effect of Sample and Vessel Size

Sample Weight, Grams	Vessel Size, Ounces	Pore Volume, Cc./Gram
5	2	0.54
10	4	0.54
25	4	0.53, av.[a]

[a] 15 runs, standard deviation = 0.006.

Film Thickness at End Point. The liquid required for the caking end point with ground sand having an average particle size of 50 microns was found to be 0.006 ml. per gram or 0.006 ml. per cc. (bulk). This corresponds to a film thickness of 0.12 micron.

Mixing and Equilibration Time. Simple methods of agitation, such as vigorous shaking by hand in a closed bottle and breaking up of lumps with a spatula, proved adequate as a means of distributing the liquid.

Although it was found that equilibration within 0.01 ml. per gram was attained with most materials in 15 minutes, about 20 hours were required for it to be complete. Because this is about the amount required for nonporous materials to reach the end point, test results obtained in a 15-minute test are subject to two very small corrections which cancel each other and, therefore, can be neglected for practical purposes.

Heat Effects. Density changes with temperature of most liquids are small enough so that temperature need be controlled only to ± 5° C.

Table III. Comparison with Nitrogen Desorption Pore Volumes on Various Fluid Catalysts

	Pore Volume (Cc./Gram, As-Run Basis)	
	Titration	Nitrogen (P/P_0 = 0.97)
Fresh silica-alumina gel A	0.53	0.55
Calcined[a] silica-alumina gel		
A	0.67	0.68
B	0.70	0.68
C	0.73	0.71
D	0.79	0.78
E	0.55	0.55
F	0.74	0.74
G	0.61	0.61
Equilibrium[b] silica-alumina gel		
A	0.18	0.16
B	0.33	0.32
C	0.39	0.38
Calcined[a] molybdena-alumina		
A	0.30	0.28
B	0.44	0.43
C	0.30	0.27
D	0.28	0.26
E	0.30	0.27

[a] 1 hour at 1100° F. in air.
[b] From commercial catalytic crackers.

PROCEDURE

Weigh out 25 grams of each sample and transfer to a 4-ounce screw-top bottle (1¾ inches in diameter × 4 inches). It is advisable to run several samples at a time. Add water rapidly from a buret in an amount slightly less than the expected pore volume. For fresh catalyst with a pore volume of about 0.70 cc. per gram, add 15 cc. and screw on lid. Shake the bottle vigorously for about 20 seconds and cool by holding under a water tap until the temperature is about 25° C. Wipe the bottle dry and break up any lumps with a spatula and/or by vigorous shaking.

Add water in 0.2-ml. increments. Between additions, break up the lumps with a spatula, then shake vigorously for 15 seconds or more. Rap the bottle against a hard surface several times, then rapidly turn it on end.

If the powder does not flow freely on upending, the end point has been reached. That is, it sticks to the bottle so as to cover the bottom completely for a period of 2 seconds or more. Repeat the rapping step if results are in doubt. When several samples are run one after the other, the elapsed time is about 15 minutes. If it is shorter than this, check the end point after 15 minutes have elapsed from the start of water addition.

$$\text{Pore volume (cc./gram)} = \frac{\text{ml. water to end point}^a}{\text{weight of sample in grams}}$$

For exact results, longer equilibration time (about 24 hours) should be used. A correction should be applied for liquid required to reach the end point for a nonporous material of the same size.

RESULTS

The method has been applied to many fluid catalyst samples, including used and fresh cracking and hydroformer catalysts. Results obtained are compared in Table III with those obtained by the conventional nitrogen desorption method.

The comparative results lead to the conclusion that the two methods give values within experimental error on fresh synthetic silica-alumina commercial cracking catalysts, and that it is reasonable to think that macroporosity is negligible. In the case of some equilibrium catalysts, titration pore volumes are slightly higher, possibly indicating a small amount of macroporosity. Hydroformer catalyst also appears to have titration pore volumes in excess of nitrogen values and to have about 0.02 cc. per gram macroporosity. The standard deviation of check results on cracking catalysts in this laboratory is less than 0.01 cc. per gram.

SOURCES OF ERROR

Factors which affect reproducibility, other than weighing and buret errors, are the following: (1) temperature of sample at end point different from that of titrating liquid; (2) loss of water by evaporation or other means; (3) technique and judgment of operator in running the test, particularly with regard to the end point; and (4) particle size distribution of sample.

The error which results because water in the pores differs in density from water in the buret is not serious, unless there is a marked difference in temperature. A difference of 10° C. in water temperature changes the density by only 0.3%.

Loss of water by evaporation is appreciable only if the sample warms up considerably, owing to heat of wetting and is not stoppered and cooled.

In regard to operator technique and judgment, it is believed that this will normally affect the results not more than 0.01 cc. per gram. Factors which have a small effect on results are the degree of shaking and rubbing out of lumps, and the time taken to upend the sample. If lumps are incompletely eliminated by rubbing or shaking, it may lead to pore volume values which are about 0.01 cc. per gram high. Overly slow upending of the sample has the same effect.

The amount of water necessary to bring about caking after pores are filled is, of course, a function of particle size. However, this quantity is so small for fluid catalysts which almost invariably have about 90% by weight in the particle size range from 15 to 200 microns, that minor differences in this correction factor have a negligible effect on the measured pore volume.

If very fine catalyst such as obtained from a Cottrell separator

[a] From change in buret reading or weight gain from water addition divided by water density at final temperature of sample.

is used, it is possible to obtain caking as defined above without any liquid addition. This caking tendency of very fine catalyst can be eliminated by addition of coarse, nonporous material such as sand, so that the method probably can be applied to very fine porous powders if necessary. Experience with these materials is limited, however. Tests can be carried out on coarse material, either ground or unground. Pores larger than the particle size will be eliminated by grinding, so that tests on the grind might give values lower than the total porosity prior to grinding.

PARTICLE AND SKELETAL DENSITY DETERMINATION

Because particle density (density of particles when pores are filled with fluid medium) determines elutriation and sedimentation behavior, it is important in regard to stack losses from commercial fluid catalyst units, as well as particle size measurement. At the present time, it is usually determined from pore volume and skeletal density (density of solid if nonporous), using the following relation for density in a medium of negligible density such as air.

$$d_p = \frac{d_s}{1 + V_p d_s}$$

where d_p = particle density, gram per cc.
d_s = skeletal density, gram/cc.
V_p = pore volume, cc. per gram

However, both d_s and V_p may be a function of temperature, pretreatment, and the like, which affect water sorption and shrinkage so that particle densities calculated from pore volumes and skeletal density obtained under other conditions may be in error. A direct measure of particle density under conditions of interest seems preferable. For example, in determining particle size distribution by the Roller method (*8*), particle densities calculated from pore volume and skeletal densities obtained under other conditions cannot be correctly applied.

To accomplish the desired objective, the sample under conditions of interest—e.g., material from a Roller test, which normally has a high water content because of use of wet air to eliminate electrostatic effects—is first tested for pore volume as described above. More water is then added to form a slurry and the sample transferred to a volumetric flask or pycnometer of known volume and tare (100 ml. is suitable for a 25-gram sample). The bottle is then filled and accurately weighed and the temperature of the mix is measured. To eliminate entrapped air, the bottle should be subjected to a swirling motion or brief evacuation prior to complete filling. Particle density can then be calculated as follows:

$$d_p = \frac{\text{sample weight}}{\text{flask vol.} - \dfrac{\text{final wt.-tare wt.- sample wt.}}{\text{water density at temperature}} + \text{vol. added to pore vol. end point}}$$

Skeletal density is given by:

$$d_s = \frac{\text{sample weight}}{\text{flask vol.} - \dfrac{\text{final wt.-tare wt.- sample wt.}}{\text{water density at temperature}}}$$

The skeletal density data obtained (Table IV) were in good agreement with data obtained in the conventional manner (*1*) by the Stamford Analytical Laboratory, using isopropyl alcohol as the medium.

Table IV. Density Data on Calcined Silica-Alumina Catalyst

Method	Pore Volume, Cc./Gram	Density, Gram/Cc.	Skeletal Density[a], Gram/Cc.
Water titration	0.675	0.916	2.45
Water titration (check determination)	0.670	0.913	2.44

[a] A value of 2.46 grams per cc. was obtained by the conventional method (*1*) using isopropyl alcohol at 30° C.

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Oxidation of Oxalic Acid in Glacial Acetic Acid with Cerium(IV)

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A solution of ammonium hexanitratocerate in glacial acetic acid may be used as a volumetric reagent, provided it is kept in an amber flask and standardized each day. Oxidations must be carried out in the presence of perchloric acid, so that the rate will be reasonably rapid. The oxidation of oxalic acid in glacial acetic acid requires 2 equivalents of cerium(IV) and yields 2 moles of carbon dioxide per mole of oxalate. Tracer studies with carbon-14 show that both moles of carbon dioxide come from the glacial acetic acid solvent. This discovery was completely unexpected. Many oxygenated organic molecules do not interfere in the oxidation of oxalic acid.

IN SPITE of the fact that nonaqueous acidimetry has undergone tremendous development in recent years, nonaqueous redoximetry has received comparatively little attention (*9, 10*). Oxidations of organic substances with cerium(IV) salts in aqueous media are not well understood, but it was thought that an investigation of similar oxidations in nonaqueous media could explain the role of water in the aqueous media. Sodium oxalate has been recommended as a primary standard for cerium(IV) solutions (*6, 7*); therefore, the initial work was on the oxidation of oxalic acid formed from sodium oxalate in glacial acetic acid solvent. Acetic acid was chosen because it is readily available in pure form

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and dissolves most oxygenated organic molecules. In attempts to study the oxidation of organic substances other than oxalic acid, it was found that the acetic acid solvent participated in the reaction. Solvent participation was also found for the oxalic acid oxidation. This unexpected result makes the oxidation mechanism in general less clear.

EXPERIMENTAL

Acetic acid used in this work was either reagent grade or analyzed reagent. No purification was required except for the stability studies, for which the acid was distilled once from chromium trioxide and once from potassium permanganate. All cerium salts were obtained from the G. Frederick Smith Chemical Co. All other chemicals were either C.P. or reagent grade. Acetic anhydride was redistilled before use. Sodium 1-carbon-14-acetate was obtained from the Chem Rad Division, Nuclear Instrument and Chemical Co., Chicago.

Cerium(IV) solutions were found to be sensitive to light. They were kept, therefore, in amber bottles, and all titrations were done using amber burets. Qualitative tests on gas evolved during oxidations were made with an Orsat-type gas analyzer.

Table I. Solubility of Cerium(IV) Salts in Acetic Acid

Salt	Normality
$Ce(SO_4)_2$	0.005
$(NH_4)_2Ce(SO_4)_3$	0.005
$Ce(OH)_4$	0.01
$(NH_4)_2Ce(NO_3)_6$	0.05

A Fisher Elecdropode (0.025 μa. per scale division) equipped with 2-cm., 18-gage platinum wire active electrodes was used for amperometric end points. A Sargent potentiometer was used for potentiometric measurements. A magnetic stirrer was used during titration; provision was made for introducing nitrogen into solutions where necessary. Counting in the radioisotope studies was done with a Nuclear scaling unit, Model 163, in conjunction with a Tracerlab windowless flow counter, SC16, fed with Matheson Geiger flow gas (helium-isobutane).

Solutions of cerium(IV) salts were prepared by saturating glacial acetic acid at 60° C. with the dried, finely powdered salt, allowing the solution to cool in the dark to room temperature, and filtering through a sintered-glass filter of medium porosity. A substance approximating ceric hydroxide was prepared by neutralizing a solution of cerium(IV) nitrate with dilute ammonium hydroxide and washing the precipitate thoroughly with water, then air drying. The solid thus obtained could well be a basic nitrate. The silver–silver chloride electrode used in this work was prepared in the usual way (*2*). Organic chemicals studied as interferences were C.P. quality and were not purified further.

Titrations were carried out by weighing portions of sodium oxalate or pipetting aliquots of a glacial acetic acid solution of sodium oxalate into 150-ml. beakers, then adding 50 ml. of glacial acetic acid and enough 70% perchloric acid to make the final mixture 1*N* in perchloric acid. The resulting solution was titrated with ammonium hexanitratocerate as described below.

For cases where the carbon dioxide was to be examined, the titration was carried out in a conical flask attached to a water condenser, which led to an absorption bulb containing standard 0.2*N* barium hydroxide. The sample solution was deaerated with carbon dioxide–free nitrogen. The carbon dioxide formed was washed by passing through water and was swept into the barium hydroxide solution with the same gas. The excess base was titrated with standard acid to determine the carbon dioxide absorbed.

Solvent participation studies were carried out using sodium 1-carbon-14-acetate in the following way. A 0.1-mc. quantity of tagged sodium acetate (about 8 mg.) was dissolved in 20 ml. of glacial acetic acid. Exactly 1 ml. of this solution was added to exactly 50 ml. of solution containing the sodium oxalate to be oxidized and to exactly 50 ml. of cerium(IV) solution, so that dilution of the carbon-14 would not occur on mixing. The required amount of cerium(IV) solution was added to the oxidation solution and the carbon dioxide caught as previously described. The barium carbonate was collected on a sintered porcelain crucible, dried, and weighed. A 50-mg. portion was transferred to a 2-cm. aluminum counting dish, and the activity was counted in the flow counter. A weighed portion of the solvent containing carbon-14 was burned to carbon dioxide in a combustion train and the activity determined in the same manner. By these procedures the counts per minute per milligram of barium carbonate from both the solvent and the oxidation were determined.

In order to prove positively that oxalic acid did not exchange with acetic acid in the presence of perchloric acid, a mixture containing cerium(III), tagged acetic acid, and sodium oxalate dissolved in 20 ml. of glacial acetic acid was allowed to stand at room temperature for 4 days. At this time the volume was reduced to a few milliliters by vacuum distillation at room temperature. The residue was taken up in water, the cerium(III) was precipitated by neutralization with dilute ammonium hydroxide, and the oxalate was isolated as calcium oxalate. After washing and drying the calcium oxalate showed no higher count than the background count for that day.

SOLUBILITY OF CERIUM(IV) SALTS IN ACETIC ACID

When this work was started, no information was available on the solubility of cerium(IV) salts in glacial acetic acid. Saturated solutions of ceric sulfate, ammonium trisulfatocerate, ceric hydroxide, and ammonium hexanitratocerate were prepared. The first three were analyzed by adding 2 ml. of 70% perchloric acid to 50-ml. portions and titrating with standard iron(II) perchlorate in glacial acetic acid (*3*). Iron(II) perchlorate can not be used in the presence of nitrate, however, and the solution of ammonium hexanitratocerate was analyzed by titration of sodium oxalate as described below. The approximate solubilities found are listed in Table I. Exact solubilities have little meaning because solutions are not stable.

In an attempt to improve the solubility of ceric hydroxide, the dry solid was moistened with mineral acids including sulfuric, perchloric, and nitric. In all cases the solubility in acetic acid was increased, but the resulting solution completely decomposed in a few hours, as shown by the loss of color and oxidizing power.

From these experiments it was concluded that only ammonium hexanitratocerate was worth further investigation.

Table II. Effect of Acid on Potential of Cerium(III)–Cerium(IV) Couple in Acetic Acid

Acid	*N* of Acid	*E vs.* Ag-AgCl, Volts
$HClO_4$	0.25	0.99
	0.50	1.00
	1.0	1.02
	1.5	1.04
	2.5	1.06
H_2SO_4	2.5	0.81

DETECTION OF END POINTS

Three possible methods for the detection of the end point in titrations of oxalic acid with acetic acid solutions of ammonium hexanitratocerate were considered—namely, indicators, potentiometric, and amperometric with two active electrodes.

The indicator method was soon abandoned when it was found that the oxidation of oxalic acid, even in 1*N* perchloric acid, was fast but not instantaneous. Therefore, the end point was usually missed, because no indication of the vicinity of the end point could be observed.

In order to use the potentiometric method for end-point detection, information was required concerning suitable electrodes for use in 1*N* perchloric acid in glacial acetic acid, potentials for cerium systems in acetic acid, and rates of attainment of stable potentials. To study potential changes, a solution of sodium oxalate in 1*N* perchloric acid was oxidized with ammonium hexanitratocerate and an equal amount added in excess so that approximately equivalent amounts of cerium(III) and cerium(IV) were present. When the process was followed using platinum and calomel electrodes, large breaks in potential were found in the vicinity of the equivalence point, but individual values were not

Table III. Sensitivity of Amperometric End Point[a]

Potential, Mv.	$\frac{\Delta i}{\Delta Ml.}$, $\frac{\mu a.}{Ml.}$
200	8.13
225	8.75
250	9.75
275	10.5
300	10.3

[a] 39.6 mg. $Na_2C_2O_4$ titrated with 20.67 ml. 0.0290N Ce(IV).

Table IV. Reproducibility of Amperometric End Points

Ce(IV) Soln.	N of Ce(IV) Soln.
1	0.0261, 0.0259, 0.0260
2	0.0426, 0.0426, 0.0425
3	0.00913, 0.00914

reproducible and considerable variation with time was observed. This system might be used for end-point detection, but it would be of little value in measuring the cerium(III)–cerium(IV) formal redox potential. The measurements were then repeated using platinum or silver–silver chloride electrodes with different acid concentrations. At least three conclusions may be drawn from the data (Table II). First, the values have only a relative meaning, because the value for the silver–silver chloride electrode in 1N perchloric acid in glacial acetic acid is unknown. Second, the solution contains a multitude of species, and what is being measured is not clear. Third, there is a definite anion effect, because the addition of sulfuric acid to a perchloric acid solution immediately lowers the potential. As a result, these values must be looked upon as only preliminary; further work is in progress to clarify the situation. An unusual observation in the course of the work was that flakes of silver chloride fell from the surface of the electrode as it stood in acetic acid. The end point may be detected using silver–silver chloride electrodes if necessary, but the flaking is an objection.

The amperometric end point using two active platinum electrodes for titrations with cerium(IV) in aqueous medium has been noted (*8*). There was no reason to believe that the method would not work in nonaqueous media. A portion of sodium oxalate was dissolved in 1N perchloric acid in acetic acid and an equivalent amount of cerium(IV) solution was added plus 1 drop more. Cleaned platinum electrodes were inserted, and the sensitivity, microamperes per milliliter, was determined with different potentials applied (Table III). At the same time it was found that the sensitivity as well as the rate of oxidation was greater in 1N acid than in 0.5N acid. From these results an applied potential of 275 mv. was selected for all future work.

To check the reproducibility of the amperometric end point, three different cerium(IV) solutions were used to titrate aliquots of a solution of sodium oxalate in acetic acid, made 1N in perchloric acid. The results are summarized in Table IV.

STABILITY OF ACETIC ACID SOLUTIONS OF AMMONIUM HEXANITRATOCERATE

Because the slow oxidation of acetic acid by cerium(IV) in aqueous media has been reported (*1*, *4*, *6*), it was necessary to determine the stability of acetic acid solutions of ammonium hexanitratocerate.

Aqueous solutions of cerium(IV) are sensitive to light (*6*). A solution of ammonium hexanitratocerate in acetic acid with no perchloric acid present was divided into two parts. One part was placed in a clear, glass-stoppered borosilicate flask, the other in an amber, glass-stoppered borosilicate flask. Both were stored on the bench top at room temperature (23° to 26° C.) with no additional protection from light. Portions were removed periodically from each flask and used to titrate aliquots of a known solution of sodium oxalate in acetic acid. The results are summarized in Table V. The times noted are within ±0.5 hour; therefore, the results represent crude rate data. Obviously, cerium(IV) solutions in acetic acid must be kept in dark bottles and titrations performed using amber burets to minimize decomposition by light. It is also apparent that frequent standardization is necessary.

The effect of perchloric acid on the stability of acetic acid solutions of cerium(IV) was also determined. For this study an amount of perchloric acid to yield the desired concentration was added to the cerium(IV) solution in an amber flask. Aliquots were removed periodically and added to excess sodium oxalate in 1N perchloric acid, and the excess was titrated with another standardized cerium(IV) solution. From the results shown in Table VI, it can be seen that the oxidizing power of cerium(IV) solutions in acetic acid in the presence of perchloric acid decreases very rapidly. Therefore, under these conditions excess techniques are not applicable and only small amounts of cerium(IV) may be added during direct titrations. These limitations restrict the use of acetic acid solutions of cerium(IV) considerably.

Table V. Light Sensitivity of Acetic Acid Solutions of Cerium(IV)

Time, Days	N of Ce(IV) Soln. Clear flask	Amber flask
0	0.0260	0.0260
1	0.0241	0.0255
2	0.0230	0.0253
3	0.0185	0.0248
5	0.0134	0.0221

Table VI. Stability of Acetic Acid Solutions of Cerium(IV) Containing Perchloric Acid

Time, Minutes	N of Ce(IV) Solution 0.5N $HClO_4$	0.75N $HClO_4$	1.0N $HClO_4$
0	0.0405	0.0318	0.0306
20	0.0385	0.0372	0.0254
50	0.0360	0.0244	0.0186
110	0.0304	0.0190	0.0146

POSSIBLE REDUCTANTS IN ACETIC ACID

At one point in this work it appeared desirable to have a reducing agent available to remove cerium(IV) quickly by reduction to cerium(III). The various substances tried included arsenious oxide, sodium arsenite, stannous chloride, ferrous sulfate, Mohr's salt, Oesper's salt (ferrous ethylenediaminesulfate tetrahydrate), and ferrous chloride. All these were either only sparingly soluble in acetic acid or underwent a reaction with the solvent. Sodium nitrite was soluble and readily oxidized, but a solution of sodium nitrite in acetic acid evolved a colorless gas after only a few minutes. Iron(II) perchlorate was found to be suitable under certain conditions (*8*). No completely satisfactory reducing agent is known for use in acetic acid.

OXIDATION OF OXALIC ACID

When sodium oxalate is dissolved in acetic acid and titrated with ammonium hexanitratocerate, the rate of oxidation is slow. If the solution is made 1N in sulfuric acid, the rate increases a little. If the solution is made 1N in perchloric acid, the rate becomes fast but not instantaneous. This emphasizes the need for the use of perchloric acid in acetic acid solution.

At the time the work was started, it was assumed that 2 equivalents of oxidizing agent would be consumed per mole of sodium oxalate and that 2 moles of carbon dioxide would be formed as the sole product. After some preliminary experiments with other

compounds, it was clear that the stoichiometry of the oxalate oxidation had to be checked. The cerium(IV) content of a sample of ammonium hexanitratocerate was determined by aqueous reduction in the usual way, and a weighed portion was dissolved in acetic acid to yield a solution of known concentration. Weighed portions of sodium oxalate were dissolved in acetic acid which was 1*N* in perchloric acid and titrated with the cerium(IV) solution to an amperometric end point. The carbon dioxide evolved was absorbed in barium hydroxide, and the excess barium hydroxide was titrated with standard acid. The results in Table VII show that the stoichiometry appears to be normal.

Later experiments suggested that the acetic acid solvent participated in cerium(IV) oxidations. The work described above was repeated with the addition of sodium 1-carbon-14-acetate. The carbon dioxide formed was isolated as barium carbonate and counted. The specific activity of the barium carbonate from the acetic acid solvent was 2.54 (Table VIII). However, only one carbon atom in the acetic acid was labeled; therefore, the specific activity of barium carbonate per carboxyl group is 5.08. Because the specific activity of the barium carbonate from the oxidation is 5.45, all the carbon dioxide isolated came from carboxyl group carbon and 2 moles of acetic acid participate per mole of oxalic acid. None of the carbon dioxide isolated can come from the oxalic acid which has reacted.

Table VII. Oxidation of Oxalic Acid in Acetic Acid

[0.0290*N* Ce(IV)]

Mg. $Na_2C_2O_4$	Mmole. CO_2 per Mmole. $Na_2C_2O_4$	Meq. Ce(IV) per Mmole. $Na_2C_2O_4$
74.2	2.08	2.01
70.7	1.96	2.03
78.6	2.03	0.99

Table VIII. Specific Activity of Barium Carbonate

Sample	Mg. $BaCO_3$	Counts/Min., Gross	Counts/Min., Net	Counts/Min. per Mg. $BaCO_3$
Background		36.9		
Acetic acid	35.4	127.2	90.3	2.54
Sodium oxalate	50.0	310.4	273.5	5.45

When solvent participation was found, the question of the mechanism was considered briefly. If the oxidation proceeded via free methyl radicals formed by oxidation of the solvent, methane or ethane would be expected as a side reaction product (*4, 5, 11, 12*). The gases formed on a large scale oxidation were swept on a stream of carbon dioxide into 40% potassium hydroxide solution in a gas buret. No measurable amount of insoluble gas was found, indicating that hydrocarbon formation was not an important process. Tests for the presence of peroxides in the oxidation mixture were inconclusive. At this time the oxidation mechanism is unknown. Further work is in progress to explain the participation of the acetic acid solvent in the oxidation.

EFFECTS OF OTHER SUBSTANCES ON OXIDATION OF OXALIC ACID

Because oxalic acid could be oxidized at a rapid rate with cerium(IV) in acetic acid which was 1*N* in perchloric acid, and because test tube experiments showed that many oxygenated organic molecules were oxidized very slowly, it appeared that oxalic acid might be titrated in the presence of these molecules. For these experiments definite quantities of sodium oxalate and impurity were added to 1*N* perchloric acid in acetic acid and titrated to the amperometric end point with cerium(IV). From the results in Table IX it is seen that polyols and tartaric acid yield no end points. Perchloric acid plus acetic anhydride in acetic acid should be an excellent acetylating mixture; therefore, acetic anhydride was added to the polyols, and some improvement was found. The addition of acetic anhydride to ethyl alcohol, however, decreases the accuracy. No experimental evidence is available concerning the cause for this effect. In general it may be concluded that oxalic acid may be determined in the presence of a number of oxygenated molecules.

Table IX. Effect of Oxygenated Impurity on Oxidation of Oxalic Acid

Added Impurity	Amount of Impurity	Mg. $Na_2C_2O_4$ Taken	Mg. $Na_2C_2O_4$ Found
Sodium formate	50 mg.	74.4	74.6
		59.3	59.3
Acetic anhydride	3.0 ml.	53.9	53.9
		66.5	66.6
Ethyl alcohol	2.0 ml.	51.7	51.6
		36.7	36.9
Ethyl alcohol, plus acetic anhydride	1.0 ml., 3.0 ml.	38.9	36.9
	1.0 ml., 3.0 ml.	52.6	50.4
Isopropyl alcohol	2.0 ml.	41.7	41.6
		42.6	42.5
Formaldehyde	50 mg. $(CH_2O)_3$	55.6	55.9
		36.7	36.8
Benzaldehyde	0.5 ml.	No end point	
	0.2 ml.	35.1	34.9
		50.1	50.3
Acetone	2.0 ml.	48.5	48.3
		53.7	53.6
Glycerol	0.5 ml.	No end point	
Glycerol, plus acetic anhydride	0.5 ml., 3.0 ml.	45.4	45.6
	0.5 ml., 3.0 ml.	74.4	74.9
Ethylene glycol	0.5 ml.	No end point	
Ethylene glycol, plus acetic anhydride	0.5 ml., 3.0 ml.	53.8	54.2
	0.5 ml., 3.0 ml.	38.1	38.7
Sucrose	100 mg.	No end point	
	50 mg.	No end point	
Sucrose, plus acetic anhydride	50 mg., 3.0 ml.	No end point	
Tartaric acid	50 mg.	No end point	
Tartaric acid, plus acetic anhydride	50 mg., 3.0 ml.	No end point	

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Analysis of Samples Containing Uranium, Niobium, and Zirconium

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The importance to the atomic energy program of reasonably rapid, simple, and accurate methods for the analysis of mixtures containing uranium, niobium, and zirconium prompted an investigation of polarographic and amperometric procedures for this purpose. Mixtures containing uranium and zirconium can be analyzed by polarographing an aliquot of a 10% sulfuric acid solution between 0.0 and −0.5 volt to determine uranium, and titrating another aliquot amperometrically with standard cupferron solution at −1.0 volt to determine zirconium. In mixtures containing uranium, niobium, and zirconium, the zirconium is separated as the phosphate from hydrogen peroxide–sulfuric acid solution, and the suspended phosphate is titrated amperometrically with cupferron. The uranium and niobium are determined simultaneously from the polarogram of their solution in concentrated hydrochloric acid. Errors are about 1% for uranium and niobium, and about 0.5% for zirconium. The procedure was successfully applied to the assay of a niobium ore.

BECAUSE of the relatively common natural occurrence of uranium, zirconium, and niobium, as well as their occurrence in mixtures useful for the utilization of atomic energy, it seemed highly desirable to develop fairly rapid and simple procedures for the analysis of samples containing uranium and zirconium, and uranium, niobium, and zirconium.

Hafnium, while not specifically studied, would follow zirconium in the proposed schemes of analysis. Titanium is separated from zirconium and hafnium by the phosphate–hydrogen peroxide isolation of the latter two elements. Tantalum produces no polarographic wave under the conditions used to determine niobium and may thus be present in considerable quantities without causing any interference.

APPARATUS AND REAGENTS

Apparatus. A Sargent Model XXI Polarograph was used in conjunction with a thermostated H-type cell containing a saturated calomel reference electrode (*10*). Potentials were checked with a Type K potentiometer. The capillary used had an $m^{2/3}t^{1/6}$ value (25° C., open circuit, distilled water) of 2.04 mg.$^{2/3}$ sec.$^{-1/2}$.

Reagents. A standard uranium solution (0.0398 millimole or 9.47 mg. of uranium per milliliter) was prepared by dissolving reagent grade uranyl nitrate in sulfuric acid and heating until sulfur trioxide was evolved. The resultant solution was diluted to contain 10% sulfuric acid by volume; it was standardized by reduction with zinc amalgam, aerated for 5 minutes, and titrated with standard permanganate. Uranium(IV) solutions were prepared daily from the uranium(VI) solution by reduction with zinc amalgam.

A standard niobium solution was prepared by dissolving reagent grade potassium niobate in water, adding 50 ml. of saturated oxalic acid solution, warming the resulting suspension gently until solution was complete, and then adding 5 ml. of hydrochloric acid. This solution was diluted to 250 ml. and standardized gravimetrically (*17*); it contained 0.0124 millimole (1.05 mg.) of niobium per milliliter and remained unchanged in composition throughout the investigation. When this solution was used, the oxalate present was first destroyed by fuming with sulfuric acid and then adding, as necessary, a few drops of dilute permanganate.

Standard zirconium solution, prepared by dissolving the pure metal in boiling concentrated hydrochloric acid, contained 0.0757 millimole (6.905 mg.) of zirconium per milliliter.

Standard cupferron solutions in oxygen-free water were prepared daily from a purified sample of cupferron (*15*). Nitrogen was purified and equilibrated in the usual fashion (*15*). All other chemicals were reagent or C.P. grade and were used without further purification.

EXPERIMENTAL

Polarographic Determination of Group IVB Metals. The polarography of titanium has been summarized by Kolthoff and Lingane (*9*); titanium is generally determined in strongly acidic solution by reduction of titanium(IV) to titanium(III). No wave is observed for the titanate ion in strongly alkaline solution. In solutions containing high concentrations of oxalate, tartrate, or citrate, titanium gives a well-defined wave over the pH range from 0.5 to 11.8 (*19*).

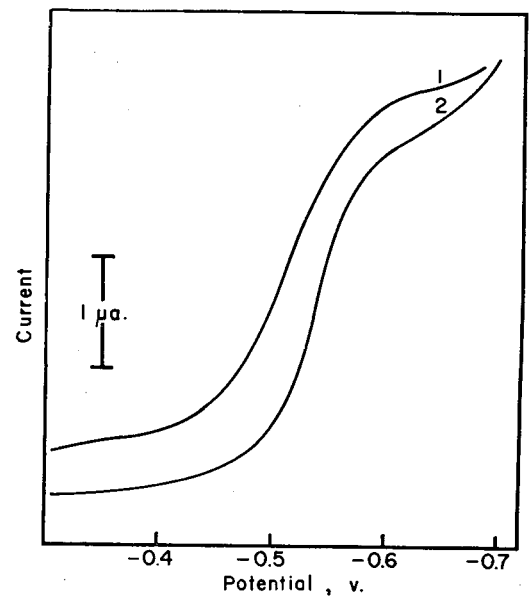

Figure 1. Polarographic curves for 1.16m*M* niobium(V)

1. **In 12*M* hydrochloric acid**
2. **In 9*M* sulfuric acid**

The polarographic reduction of zirconium in 1*M* hydrochloric acid solution containing potassium chloride has been reported (*12*); however, it is preceded by the reduction of hydrogen and is therefore of little use. More recently the polarographic reduction of zirconium in methanol solutions has been described (*1*) and may prove to be useful after further study. The polarographic reduction of hafnium and thorium has not been achieved as yet.

Reaction of Group IVB Metals with Cupferron. Previous studies (*5, 6, 14, 15*) have established the optimum conditions for the rapid quantitative precipitation of the Group IVB metals with cupferron. The amperometric titration of titanium, zirconium, and hafnium with cupferron has been described (*5, 15*).

Polarography of Niobium and Tantalum. Niobium gives a polarographic wave in 1*M* nitric acid (*20*); although the limiting current is proportional to niobium concentration, the wave is actually due to the catalytic reduction of nitrate ion in the

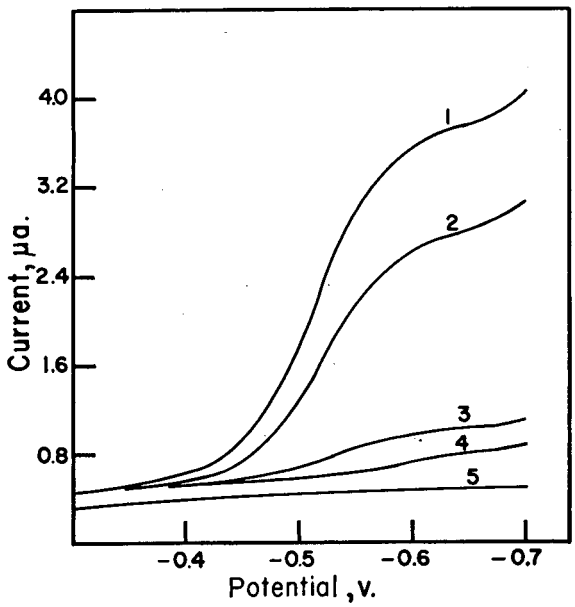

Figure 2. Effect of hydrochloric acid concentration on polarographic wave of 1.16m*M* niobium(V)

1. 12*M* hydrochloric acid
2. 9.6*M* hydrochloric acid
3. 7.2*M* hydrochloric acid
4. 4.8*M* hydrochloric acid
5. Residual current in 12*M* hydrochloric acid

presence of niobium (*3*). In concentrated hydrochloric acid niobium(V) is reduced to niobium(IV) by a reversible 1-electron process ($E_{1/2}$ of −0.455 volt *vs.* S.C.E.; the wave height is proportional to concentration and reproducible in 9- to 12*M* hydrochloric acid); a second wave appears but merges with the hydrogen discharge wave (*2, 4*). The polarographic reduction of niobium in sulfuric acid solution has also been reported (*11*), but details are not available. Consequently, a brief study was made of the polarographic behavior of niobium in sulfuric and hydrochloric acid solutions.

Niobium gives a well-defined wave in very strongly acidic solutions of both sulfuric and hydrochloric acids (Figure 1); the wave in hydrochloric acid is not quite as sharp but is better defined because of the larger diffusion current plateau. The minimum hydrochloric acid concentration necessary to obtain the full wave height is about 11.6*M* (Table I, Figure 2). An increasing Tyndall effect appears on decreasing the hydrochloric acid concentration; in fact, after standing for several hours, a considerable amount of niobic acid had precipitated from the 7.2*M* and 4.8*M* hydrochloric acid solutions. All of the polarographic test solutions were prepared at the same time and were run in order, starting with the 12*M* solution. About 15 minutes was required for each run, so that the last sample (4.8*M*) would have been run about 2 hours after preparation. The 12*M* solution was rerun after the last sample had been run; no decrease in current was found. It therefore appears that the decrease in the niobium wave is the result of the hydrolytic formation of niobic acid, even in 9.6*M* hydrochloric acid.

Under the conditions described, tantalum does not produce a polarographic wave.

Polarographic Behavior of Uranium in Presence of Cupferron. It had previously been noticed that, when cupferron was added in sufficient excess to a solution of uranyl ion in 10% sulfuric acid, a bright orange color resulted, indicating that reaction had occurred. Consequently, solutions of uranyl sulfate in 10% sulfuric acid were titrated amperometrically with standard cupferron solution at a potential of −0.4 volt *vs.* S.C.E. (Figure 3). The curves obtained indicated no relationship between the amount of uranium(VI) present and the volume of cupferron

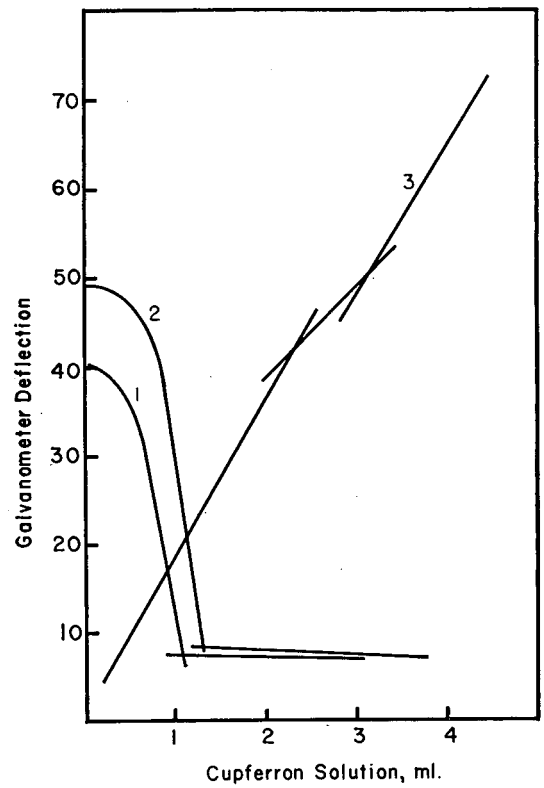

Figure 3. Amperometric titration of uranium(VI) and uranium(IV) with 0.0672*M* cupferron in 50 ml. of 2*M* sulfuric acid

1. 0.0769 millimole of uranium(VI)
2. 0.1990 millimole of uranium(VI), run at one half the sensitivity of curve 1
3. 0.0199 millimole of uranium(IV)

Table I. Effect of Hydrochloric Acid Concentration upon Height of Polarographic Wave of 1.16m*M* Niobium(V)

HCl Concn., *M*	Niobium, *id*, μa.	HCl Concn., *M*	Niobium, *id*, μa.
12.0	3.95	10.8	3.60
11.6	4.00	9.6	2.80
11.5	3.90	7.2	0.65
11.4	3.80	4.8	0.35

Table II. Half-Wave Potentials and Currents of Uranium and Cupferron in Presence of Each Other in 2*M* Sulfuric Acid

(Uranyl Concentration 4 × 10⁻³*M*)					(Cupferron Concentration 4.5 × 10⁻³*M*)		
Cupferron			Uranium		Uranyl	Cupferron	
M × 10³	−$E_{1/2}$, volt	*id*, μa.	−$E_{1/2}$, volt	*id*, μa.	*M* × 10³	−$E_{1/2}$, volt	*id*, μa.
0.00			0.18	24.0	0.00	0.52	27.8
0.64	0.62	6.6	0.19	22.4	0.08	0.61	27.8
0.92	0.63	8.8	0.18	21.6	0.1	0.65	27.6
1.28	0.64	11.6	0.21	20.4	0.2	0.65	28.0
1.60	0.66	13.8	0.29	19.8	0.4	0.65	28.0
1.92	0.66	14.6	0.40	20.6	0.8	0.66	28.6
2.56	0.66	18.0	0.48	21.4			
3.20	0.66	20.0	0.52	22.0			

required to reach the apparent end point. Consequently, the rapid decrease in uranium current found on adding cupferron was investigated polarographically by observing the effects of varying uranium or cupferron concentrations at a constant concentration of the other.

The results of this study, summarized in Table II and Figure 4, indicate that the apparent decrease in current is due to the shifting of the uranium wave to more negative potentials as cupferron is added; this shift in $E_{1/2}$ of uranyl ion is not accompanied by an appreciable decrease in i_d. The cupferron wave is also slightly shifted to more negative potentials. This shift of waves occurs at cupferron concentrations much too low to cause complete complexation of the uranium present; therefore, it should be accompanied by a wave split. However, no waves other than the two attributable to uranyl ion and cupferron appear on the polarograms.

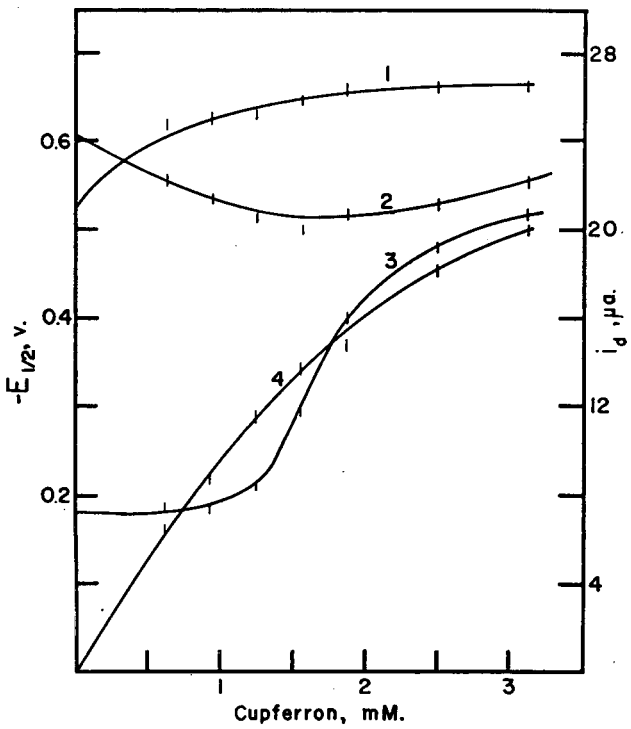

Figure 4. Half-wave potentials and diffusion currents of uranium and cupferron as a function of cupferron concentration

1. $E_{\frac{1}{2}}$ of cupferron
2. i_d of uranium(VI)
3. $E_{\frac{1}{2}}$ of uranium(VI)
4. i_d of cupferron

These studies indicated the desirability of performing titrations employing cupferron in the presence of uranyl ion at an applied potential of -1.0 volt *vs.* S.C.E., because the shift in $E_{1/2}$ of cupferron is sufficiently small to enable the wave to attain its full height at this potential. If the titration is performed at -0.84 volt (*15*), the current increase after reaching the equivalence point will not be as rapid as before, because the potential of -0.84 volt occurs on the steeply rising portion of the wave rather than on the limiting current plateau.

Recent polarographic studies on the uranium(VI)-cupferron system at lower acidities (*16*) indicate that complexation occurs in solutions approximately 0.1*M* in hydrogen ion. However, no such complexation in solutions 2*M* in sulfuric acid was found in the present investigation.

Table III. Titration of Zirconium in 10% H_2SO_4 in Presence of Uranium(IV), Uranium(VI), and Niobium(V)

Added		Zirconium, Millimole		
Species	Millimole	Taken	Found	Deviation
		0.0757	0.0758	+0.0001
		0.0757	0.0755	−0.0002
		0.0757	0.0759	+0.0002
		0.0757	0.0757	0.0000
UO_2^{++}	0.04	0.0757	0.0756	−0.0001
UO_2^{++}	0.08	0.0757	0.0756	−0.0001
UO_2^{++}	0.12	0.0757	0.0759	+0.0002
UO_2^{++}	0.20	0.0757	0.0752	−0.0005
U(IV)	0.08	0.0000	0.0000	
U(IV)	0.20	0.0000	0.0000	
U(IV)	0.04	0.0757	0.1001	+0.0244
U(IV)	0 04	0.0757	0.0976	+0.0219
U(IV)	0.08	0.0757	0.1172	+0.0415
U(IV)	0.08	0.0757	0.1191	+0.0434
Nb(V)	0.025	0.0757	0.0906	+0.0149
Nb(V)	0.012[a]	0.0757	0.0794	+0.0037
Nb(V)	0.025[a]	0.0757	0.0818	+0.0061
Nb(V)	0.062[a]	0.0757	0.0852	+0.0095
Nb(V)	0.025[b]	0.0757	0.0755	−0.0002
Nb(V)	0.031[b]	0.0757	0.0759	+0.0002
Nb(V)	0.062[b]	0.0757	0.0754	−0.0003
Nb(V)	0.025[c]	0.0757	0.0897	+0.0142
Nb(V)	0.025[c]	0.0757	0.0888	+0.0131

[a] Niobium precipitated by hydrolysis; resulting suspension titrated.
[b] Zirconium separated as phosphate in presence of H_2O_2.
[c] Zirconium separated as phosphate in absence of H_2O_2.

Attempted Amperometric Titration of Uranium. The amperometric titration of uranium(IV), which is precipitated quantitatively by cupferron (*6*), was attempted. From a typical titration curve (Figure 3), it is apparent that a considerable excess of cupferron must be present to initiate precipitation; direct titration of uranium(IV) is not possible. The instability of cupferron in acid solution effectively precludes a back-titration procedure. Moreover, the data in Table III show that it is not possible to titrate zirconium in the presence of uranium(IV) without precipitating the uranium. The fact that the uranium is more nearly completely precipitated when the uranium to zirconium ratio is small, rather than when it is large, also indicates that this is a case of coprecipitation. Because uranium(IV) is readily oxidized to uranium(VI), its presence does not affect the zirconium determination.

Polarography of Uranium in Concentrated Hydrochloric Acid. Kolthoff and Harris (*7*, *8*) have described the polarographic behavior of uranium(VI) in 0.01 to 0.2*M* and in 1.0 to 2.0*M* hydrochloric acid solutions. The fact that niobium gave a well-defined polarographic wave at moderately negative potential in 12*M* hydrochloric acid solution prompted a brief study of uranium in this medium. At 25.0° C., uranium(VI) gave a well-defined wave, whose $E_{1/2}$ cannot be precisely evaluated because the toe of the wave merges with the anodic wave from mercury. Values of i_d/C were 9.2 ± 0.1, which corresponds to I equal to 4.51.

Prolegomena to the Analysis of Mixtures of Uranium, Niobium, and Zirconium. It was first thought that the best method for analyzing mixtures of niobium and zirconium would be to determine the sum of zirconium and niobium by titration with cupferron, and then determine one of these elements. However, titration of niobium under various conditions revealed that, although niobium is quantitatively precipitated, it does not form a stoichiometric cupferrate. The ratio of cupferron to niobium varied from 2.7 to 3.8, depending upon the conditions of precipitation—e.g., acidity and rate of reagent addition. This indicates formation of a mixture of niobium cupferrates. Typical titration curves of niobium with cupferron in 0.1*M* and in 2*M* (10%) sulfuric acid (Figure 5) show a sharp initial increase in current when the first milliliter or so of cupferron solution is added. However, when zirconium and niobium are titrated in the presence of each other, the niobium coprecipitates, so that satisfactory results for zirconium cannot be obtained (Table III).

Table III also shows the effects of various means of separating zirconium and niobium upon the titration results. Hydrolysis of

niobium to niobic acid and titration of the resulting suspension is an attractive possibility because no separations are involved; however, an appreciable positive error results when this method is applied. However, it is possible to titrate accurately a suspension of zirconium phosphate with cupferron (*15*); the method finally developed uses prior separation of zirconium by precipitation as the phosphate in the presence of hydrogen peroxide, followed by titration of the resuspended phosphate. The fact that hydrogen peroxide is necessary to prevent precipitation of considerable quantities of niobium is also shown in Table III.

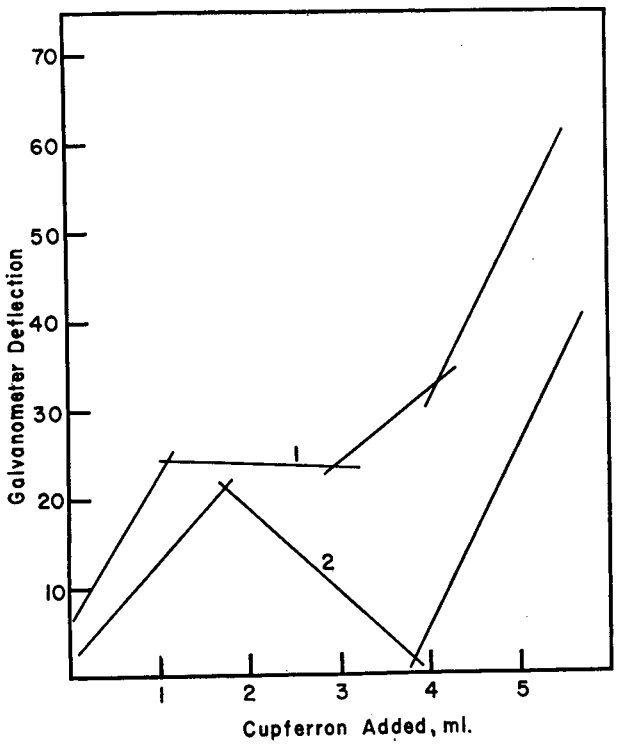

Figure 5. Amperometric titration of 0.0730 millimole of niobium(V) with 0.0648*M* cupferron

1. In 0.1*M* sulfuric acid
2. In 2*M* sulfuric acid

The determination of uranium and niobium in admixture was achieved simultaneously by a direct polarographic method.

Calibration Data. The data in Figure 6 establish the direct proportionality to concentration of the wave heights of niobium(V) and uranium(VI) in concentrated hydrochloric acid, and of uranium(VI) in 10% sulfuric acid.

PROCEDURES

Uranium and Zirconium. Transfer a sulfuric acid solution of the sample containing the equivalent of 10 ml. of sulfuric acid (add acid if necessary) to a platinum dish, and cautiously add 10 ml. of water. While the solution is still hot, add dilute permanganate solution (about 0.001*N*) until the permanganate color persists. Add 2 ml. of concentrated hydrochloric acid and heat the solution until copious fumes of sulfur trioxide are evolved. Cool and dilute the residue to 100 ml. in a volumetric flask containing 1 ml. of 0.1% gelatin solution. Transfer a portion of this solution to the polarographic cell, and, after the removal of oxygen, record the uranium polarogram between 0.0 and −0.5 volt *vs.* S.C.E. Determine the uranium concentration in the test solution by comparing the uranium wave height (determined geometrically or using a residual current correction) with a standard curve. Calculate the amount of uranium in the original sample on the basis of the dilutions employed.

Using another aliquot of the gelatin-containing solution and the procedure previously described (*15*), titrate the zirconium amperometrically with standard cupferron solution at −1.0 volt *vs.* S.C.E.

Uranium, Niobium, and Zirconium. Transfer a solution of the sample to a 150-ml. beaker; add sufficient concentrated sulfuric acid to make the total amount of acid present 10 ml.; if oxalate is present, add 25 ml. of concentrated nitric acid. Evaporate until sulfur trioxide is evolved vigorously. Cool and dilute to 100 ml. in a volumetric flask. Pipet an aliquot of this solution (containing about 0.1 millimole of zirconium) into a 125-ml. centrifuge tube; add 50 ml. of 10% sulfuric acid, 3 ml. of 30% hydrogen peroxide, and 25 ml. of 10% sulfuric acid saturated with ammonium dihydrogen phosphate (about 25 grams per 100 ml. of acid).

Mix and centrifuge, then wash the zirconium phosphate several times by centrifugation with a solution prepared by mixing 50 ml. of the sulfuric acid–ammonium phosphate solution, 50 ml. of 10% sulfuric acid, and 3 ml. of 30% hydrogen peroxide. After washing, suspend the zirconium phosphate precipitate in about 50 ml. of 10% sulfuric acid and titrate with cupferron (*15*). Alternatively, filter the zirconium phosphate on a small rapid filter paper such as Whatman No. 41, and, after washing, suspend the paper and precipitate in 10% sulfuric acid. Pulverize the paper with a stirring rod and titrate the resulting suspension.

Measure a second portion of the original sample solution into a 150-ml. beaker, add 5 ml. of concentrated sulfuric and 25 ml. of concentrated nitric acid, and heat until sulfur trioxide is evolved. Dissolve the residue in concentrated hydrochloric acid, transfer to a 100-ml. volumetric flask containing 1 ml. of 0.1% gelatin solution, and dilute to 100 ml. with concentrated hydrochloric acid. Add a portion of the latter solution to the polarographic cell, and, after the removal of oxygen, record the polarogram.

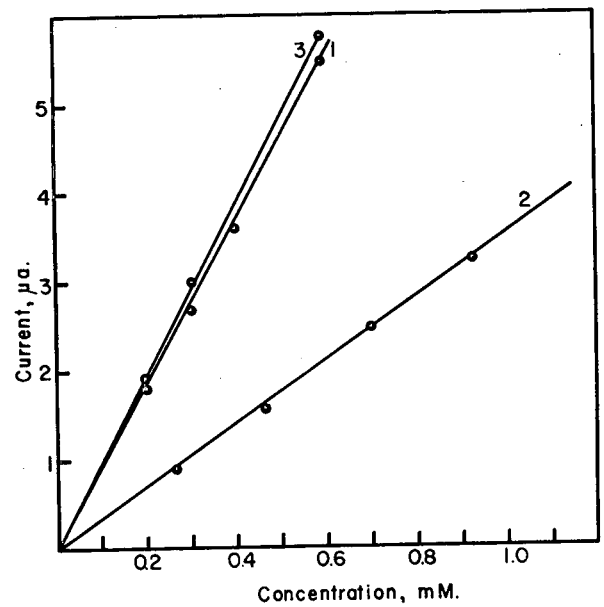

Figure 6. Calibration curves

1. Uranium(VI) in 12*M* hydrochloric acid
2. Niobium(V) in 12*M* hydrochloric acid
3. Uranium(VI) in 2*M* sulfuric acid

Determine the height of the uranium wave between −0.3 and −0.4 volt *vs.* S.C.E. by comparison with the residual current curve of concentrated hydrochloric acid. Determine the niobium wave height directly without correction for the residual current polarogram. From the i_d values thus obtained, determine the niobium and uranium concentrations from standard curves (Figure 6).

Niobium Ores. Fuse the sample with potassium carbonate in a platinum dish; dissolve the residue in water with the aid of a little potassium hydroxide (*18*). After solution is complete, filter through a medium glass fritted funnel and transfer to a 400-ml. beaker. Add 10% sulfuric acid dropwise until the solu-

Table IV. Analysis of Uranium-Zirconium Mixtures

Sample No.	Zirconium, Millimole Taken	Zirconium, Millimole Found	Error, %	Uranium, Millimole Taken	Uranium, Millimole Found	Error, %
1	0.0303	0.0306	+0.99	0.0398	0.0391	−1.75
2	0.0303	0.0301	−0.66	0.0398[a]	0.0402	+1.01
3	0.0303	0.0300	−0.99	0.0796	0.0790	−0.75
4	0.0303	0.0301	−0.66	0.0796[a]	0.0785	−1.38
5	0.0757	0.0759	+0.26	0.0398	0.0403	+1.25
6	0.0757	0.0759	+0.26	0.0398[a]	0.0393	−1.51
7	0.0757	0.0751	−0.79	0.0796	0.0801	+0.63
8	0.0757	0.0755	−0.26	0.0796[a]	0.0792	−0.50
		Av.	±0.6			±1.1

[a] Added as U^{+4} and oxidized to UO_2^{++} with permanganate during analysis.

Table V. Analysis of Mixtures of Uranium, Niobium, and Zirconium

Sample No.	Zirconium Millimole Taken	Zirconium Millimole Found	Error, %	Niobium Millimole Taken	Niobium Millimole Found	Error, %	Uranium Millimole Taken	Uranium Millimole Found	Error, %
1	0.755	0.755	−0.3	0.464	0.458	−1.3	0.199	0.202	+1.5
2	0.757	0.759	+0.3	0.464	0.461	+0.6	0.199	0.199	0.0
3	0.757	0.754	−0.4	0.464	0.470	+1.3	0.398	0.392	−1.5
4	0.757	0.754	−0.4	1.160	1.170	+0.9	0.398	0.393	−1.3
5	0.757	0.761	+0.5	1.160	1.143	−1.5	0.597	0.604	+1.2
6	0.757	0.751	−0.8	1.160	1.156	−0.3	0.597	0.600	+0.5
		Av.	±0.4			±1.0			±1.0

tion is acidic; then add 5 ml. of concentrated sulfuric and 5 ml. of concentrated hydrochloric acid (the latter reduces any manganese oxidized during the dissolution process). Evaporate until sulfur trioxide is evolved; cool and dilute to 100 ml. with concentrated hydrochloric acid in a volumetric flask containing 1 ml. of 0.1% gelatin solution. Introduce the resulting test solution into the polarographic cell, and record the polarogram after the removal of oxygen. From the i_d obtained and a standard curve, calculate the niobium concentration and then the niobium percentage.

DISCUSSION

Mixtures Containing Uranium and Zirconium. The results of the analysis of mixtures of both oxidation states of uranium with zirconium are given in Table IV. These results were obtained by the procedures described, using direct polarographic determination of the uranium as uranyl ion and amperometric titration of the zirconium in the presence of uranyl ion. The errors, ±0.6% for zirconium and ±1.1% for uranium, are of the order of magnitude expected from the methods used. Although the sample ranges cover only 0.03 to 0.075m*M* zirconium and 0.04 to 0.08m*M* uranium solutions, these limits probably could be safely extended by a factor of 10 from either end.

Although interferences were not specifically investigated, the titration of zirconium is carried out under conditions similar to those previously used (*15*), where iron(III), vanadium(V), and large amounts of tin(IV) were found to interfere; titanium(IV), hafnium(IV), and niobium(V) would also interfere. Any ion which is reduced in sulfuric acid solution in the potential region of about −0.1 to −0.4 volt *vs.* S.C.E. would interfere in the polarographic determination of uranium.

Mixtures Containing Uranium, Niobium, and Zirconium. The results of the procedures described, when applied to mixtures of uranium, niobium, and zirconium, indicate good agreement with the theoretical values (Table V). The average errors of ±1.0, ±1.0, and ±0.5% for uranium, niobium, and zirconium, respectively, are perhaps somewhat lower than expected. Starting with a liquid sample, the procedure requires between 1 and 1.5 hours to carry out a duplicate analysis for all three elements. The sample composition can vary within rather wide limits, except that the volume of the phosphate precipitate limits the upper end of the usable zirconium concentration range.

Because the procedure employs the phosphate–hydrogen peroxide separation of zirconium, only hafnium would precipitate with zirconium. It would be determined along with zirconium by the cupferron titration.

Assays of a Niobium Ore. The polarographic determination of niobium was applied to the analysis of a niobium ore which had the following chemical analysis:

	%		%
SiO_2	3.42	Al_2O_3	0.35
FeO	9.24	TiO_2	0.62
MnO	21.42	CaO	0.99
Nb_2O_5	34.93		
Ta_2O_5	29.50		
SnO_2	0.20		

Polarographic analysis gave 35.3, 34.9, 35.4, and 34.8% niobium pentoxide, or an average value of 35.1 ± 0.3%, which is in good agreement with the known value of 34.9%. The alkaline fusion procedure was chosen for solution of the sample because it prevented the iron and titanium in the sample from dissolving. Both of these would interfere in the polarographic determination of niobium.

The small amount of tin in the ore was determined as niobium, because $E_{1/2}$ for tin(IV) in concentrated hydrochloric acid solution is almost the same as for niobium(V). If an appreciable amount of tin is present, it would be necessary to perform a preliminary separation or to correct i_d of niobium for that of the tin present; the tin in the sample could be readily determined polarographically (*13*).

The determination is rapid after solution of the sample has been achieved. However, largely because of the time necessary for complete solution, about 4 hours are required to perform an analysis.

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Continuous Measurement of Dissolved Oxygen in Water

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A method for the continuous measurement of dissolved oxygen in water has been developed, based on establishing a Henry's law equilibrium between a flowing water sample and the oxygen content of a gas at constant pressure and volume. The results are not influenced by temperature, salt content, pollution, aeration constant, barometric pressure, or the dissolved nitrogen content of water. Experimental confirmation of the method for dissolved oxygen in water is presented. The design and performance characteristics of the major components of a practical instrument are given.

CONTINUOUS measurement of dissolved oxygen has long been recognized as a practical solution to many economic and technical problems in water pollution abatement and control programs. Hence, there is an interest not only in establishing permanent dissolved oxygen gaging stations at critical points on important streams, but also in developing means for rapidly obtaining the oxygen profile of polluted surface waters.

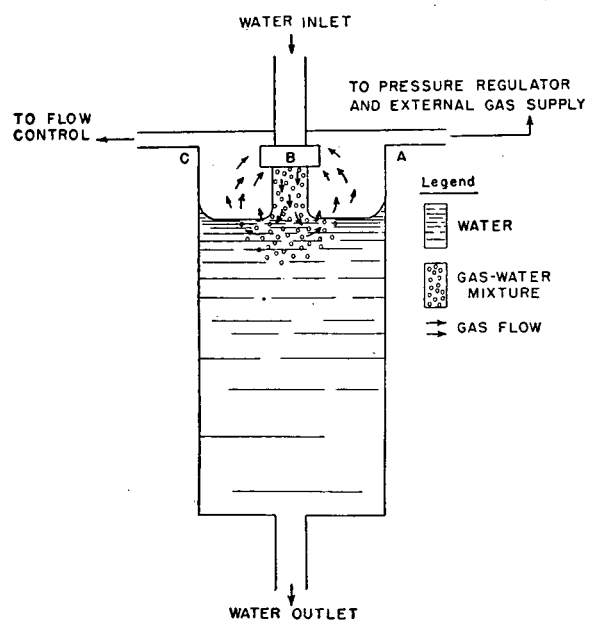

Figure 1. Typical system

The design and performance characteristics of a dissolved oxygen analyzer are related to projected uses. Performance requirements usually would call for dissolved oxygen measurements with an accuracy of about ±5% of saturation in the range from 0 to 150% of saturation for water temperatures from 0° to 35° C. General application of the method to a variety of water samples would also require accommodation of variations in suspended matter, dissolved solids, and composition and amount of pollution in the water. Design objectives for a dissolved oxygen analyzer include mobility and rapid response to changes in dissolved oxygen content, so that the instrument may be rapidly moved along the stream in a boat to obtain the oxygen profile.

Methods for the continuous measurement of dissolved oxygen in water include chemical, polarographic, and gaseous procedures. Briggs and others (*2*) described an intermittent instrument based on the Winkler method (*1*), in which a water sample is taken and a stepwise chemical test made automatically in a cycle of operations. Thayer and Robinson (*10*) reported a continuous recording instrument based on a gaseous stripping technique. Other instrumental methods have been described (*6–9*) which appear to have limited application.

A method has been developed for obtaining a continuous record of the dissolved oxygen content of water, in which the determination of dissolved oxygen is made by analysis of a gas whose composition conforms with the dissolved oxygen content of water by Henry's law (*4*). Principles are presented for the design and operation of an instrument using this method, as well as experimental confirmation of its feasibility and design information for the construction of a practical instrument.

THEORY

Principle of Operation. To conform with the convention adopted by American Public Health Association (*1*), the solubility of oxygen in water is defined as that concentration of the gas in water which is in equilibrium with oxygen in water-saturated air at 1 atm. total pressure. The solubility of oxygen in water depends on temperature, salt concentration, and the like. The actual concentration of dissolved oxygen in water, times 100, divided by its solubility as defined above is referred to in this work as per cent saturation.

Henry's law states that the equilibrium partial pressure of oxygen gas is proportional to the concentration of dissolved oxygen in water at any given temperature and salt content. This relationship is completely independent of temperature and salt content if the dissolved oxygen content of water is expressed in terms of per cent saturation as defined above. Hence, Henry's law presents an attractive basis for development of a method for continuous measurement of dissolved oxygen in water.

A typical device, referred to as an aspirator unit, for continuously maintaining a Henry's law equilibrium between the oxygen content of a small quantity of gas and the dissolved oxygen content of flowing water is illustrated schematically in Figure 1.

The system consists of a fixed volume of gas saturated with water vapor within the aspirator unit, through which water flows at a constant rate. An external gas supply at *A*, connected to the system through a pressure regulator, maintains a constant total pressure of gas in the aspirator unit. An aspirator on the water inlet at *B* intimately mixes the gas within the system with the flowing water. The gas then separates from the water and remains in the unit, cycling back through the aspirator. Water is continuously withdrawn from the bottom of the unit at a rate that maintains a constant liquid level and thus a constant gas volume. It is assumed that the volume of gas pumped through the aspirator per unit time, the time of contact between the gas bubbles and the water, the average size of the gas bubbles, and the temperature of the gas and water are all constant. Under actual conditions, physical entrainment of some of the gas in the water flowing out of the unit will occur. This can be minimized but not completely eliminated. This behavior is approximated by assuming that gas is withdrawn from the aspirator unit at a constant rate at *C*.

For this system to function as a dissolved oxygen analyzer, the gas within the aspirator unit must be in contact with a nondestructive gas analyzer that will relate the oxygen content of the gas to the dissolved oxygen content of the water.

In operation, if the actual partial pressure of oxygen gas within the unit is greater or less than that corresponding to the dissolved oxygen content of the water, then oxygen will transfer across the gas-water interface until a stable Henry's law condition is reached. Any net change in the quantity of gas within the unit during this transfer process is immediately compensated by an equivalent flow of gas in either direction through the pressure regulator at A (Figure 1).

It is important to establish whether any combination of variables will prevent the formation of a Henry's law equilibrium for oxygen. Furthermore, it is necessary to establish practical operating requirements for the instrument experimentally. Some of the variables that require more complete examination are total gas pressure, barometric pressure variations, temperature, dissolved nitrogen content of water, aeration constant of oxygen and nitrogen, and other gases dissolved in water.

Nomenclature.

J = mole fraction of oxygen in the gas that is entering or leaving the system (dry basis)
K_1 = aeration constant for oxygen
K_2 = aeration constant for nitrogen
K_3 = entrainment rate
L = maximum lag in gas composition in terms of per cent saturation
M = maximum rate of change of dissolved oxygen in water in terms of per cent saturation per unit time
N = number of moles of gas
P = constant total pressure of the system
p = $P - W$
R = molar gas constant
T = constant uniform temperature of water and gas
t = time
V = volume of gas entrained in water
v = constant volume of the system
W = partial pressure of water vapor
X = partial pressure of oxygen
Y = partial pressure of nitrogen

Let the subscripts

0 = partial pressure of oxygen or nitrogen in equilibrium with water as determined by temperature and Henry's law
1 = actual partial pressure of oxygen or nitrogen at time t
2 = initial partial pressure of oxygen or nitrogen
3 = partial pressure of oxygen or nitrogen at $t = \infty$
4 = partial pressure of oxygen or nitrogen in air saturated with water vapor at a temperature T at a total pressure of 1 atm.
x = oxygen gas
y = nitrogen gas
w = water vapor

General Relationships. It is assumed that the rate at which oxygen or nitrogen transfers across a gas-liquid interface is described by

$$\frac{dN_x}{dt} = -K_1(X_1 - X_0) \quad (1)$$

$$\frac{dN_y}{dt} = -K_2(Y_1 - Y_0) \quad (2)$$

where K_1 and K_2 are aeration constants that depend on the size and number of gas bubbles, temperature, and the flow rate of water. They are also influenced by materials dissolved in the water (*5*).

To simplify the subsequent development, it is assumed that $K_1 = K_2$. It is assumed further that the rate of gas entrainment $dV/dt = -K_3$.

Since P, v, T, and W are constant, the system must conform at all times to the following conditions of restraint:

$$\begin{aligned} Pv &= NRT \\ N &= N_x + N_y + N_w \\ p &= X_1 + Y_1 = X_2 + Y_2 = X_3 + Y_3 \end{aligned} \quad (3)$$

The total number of moles of oxygen and nitrogen gas dissolved in or removed from the water passing through the aspirator per unit time is $-K_1[(X_1 - X_0) + (Y_1 - Y_0)]$. The number of moles of oxygen leaving the system by entrainment is $-K_3X_1/RT$. The total number of moles of oxygen and nitrogen leaving the system at the same opening is $-K_3p/RT$. To maintain P constant, an equal quantity of gas must enter or leave the system through the pressure regulator at opening A. The number of moles of water involved in such exchanges need not be considered, for any loss or gain of water vapor is immediately compensated by evaporation or condensation of an equivalent amount of water in the system to maintain W constant. If J is the mole fraction of oxygen (dry basis) entering or leaving the system through the pressure regulator, then the net change per unit time in the oxygen content of the gas in the aspirator unit is:

$$\frac{dN_x}{dt} = -K_1(X_1 - X_0) - \frac{K_3X_1}{RT} + J\left[K_1(X_1 - X_0) + K_1(Y_1 - Y_0) + \frac{K_3p}{RT}\right] \quad (4)$$

The solution of Equation 4 depends on the value of J. Assuming for the moment, that the entrainment rate, K_3, is negligible, then it can be shown that if

$$X_0 < p - Y \quad (5)$$

there will be a positive flow of gas with a constant oxygen content into the aspirator unit from the external gas supply. If, on the other hand,

$$X_0 > p - Y_0 \quad (6)$$

then there will be a flow of gas with a variable oxygen content out of the aspirator unit. Finally, if the entrainment rate is significant, the situation described by Equation 6 seldom exists.

Equations 5 and 6 define dissolved oxygen regions for which the partial pressure of oxygen in the aspirator unit exhibits a specific type of functional behavior. Equation 5 represents a dissolved oxygen range less than the difference between the gas pressure and the dissolved nitrogen content of the water, whereas Equation 6 defines a range greater than this value.

Consider first the dissolved oxygen region defined by Equation 5, with a constant value of J. Because

$$\frac{dN_x}{dt} = \frac{vdX_1}{RTdt}, \quad (7)$$

then Equation 4 reduces to

$$\frac{dX_1}{dt} + AX_1 = BX_0 + C \quad (8)$$

where the constants A, B, and C are

$$A = \frac{RT}{v}K_1\left(1 + \frac{K_3}{K_1RT}\right) \quad (9)$$

$$B = \frac{RT}{v}K_1(1 - J) \quad (10)$$

$$C = \frac{RT}{v}JK_1\left(p - Y_0 + \frac{K_3p}{K_1RT}\right) \quad (11)$$

If for $t = 0$, $X_1 = X_2$, and for $t > 0$, and the dissolved oxygen content of water, X_0, is any arbitrary constant value within the region defined by Equation 5, then the solution of Equation 8 is as follows:

$$\frac{X_1 - X_3}{X_2 - X_3} = e^{-At} \quad (12)$$

where

$$X_3 = \frac{C + BX_0}{A} \quad (13)$$

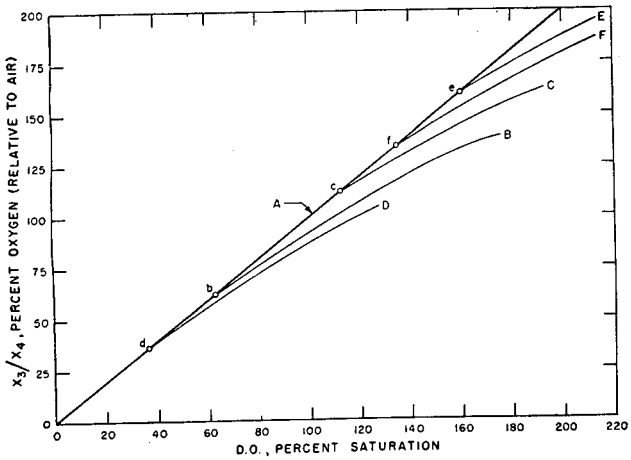

Figure 2. Oxygen content of system

$J = 0$
$K_3 \ll K_1RT$

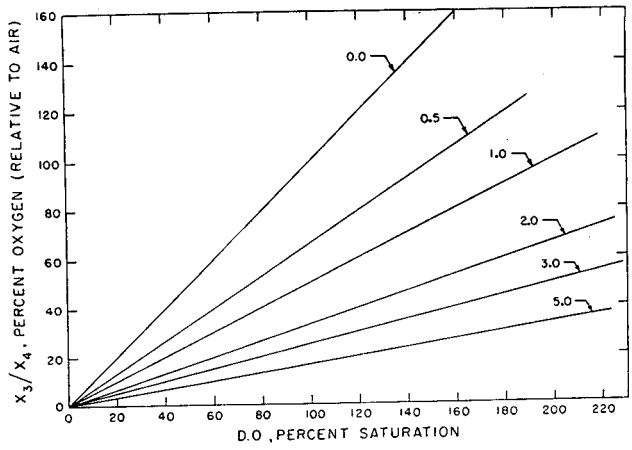

Figure 3. Oxygen content of system

Values on curves = $\frac{K_3}{K_1RT}$

That is, if initially the gas within the aspirator unit is any arbitrary oxygen-nitrogen mixture; and if, at that time taken as zero, the dissolved oxygen content of water suddenly changes to any arbitrary value consistent with Equation 5, then the partial pressure of oxygen gas will approach a final steady value, X_3, exponentially with time according to Equation 12. A final Henry's law equilibrium, independent of temperature, salt content, dissolved nitrogen content of the water, and aeration rates, which is the only result of interest here, can be attained only if the external gas supply is nitrogen ($J = 0$), and the entrainment rate is relatively small. For if $J = 0$, then $X_3 = X_0$ only if $K_3 \ll K_1RT$, because if this is not true, then

$$X_3 = \frac{X_0}{1 + \frac{K_3}{K_1RT}} \tag{14}$$

which deviates from Henry's law. With any other condition the partial pressure of oxygen in the aspirator unit approaches a final steady state value with the dissolved oxygen in the water flowing through the unit, instead of a Henry's law equilibrium, and it is further influenced by dissolved nitrogen content of the water, gas pressure, and composition of the external gas supply.

It is possible to generalize the system to include other gases in addition to oxygen and nitrogen. For the generalized system, equations of restraint similar to Equation 3 and more complicated Henry's law regions similar to Equations 5 and 6 must be established. It can be shown for the more general system that if $K_3 = 0$, then all the gaseous components in the system, except the pure gas used for the external gas supply, will conform to Henry's law in a limited concentration region. In terms of projected use, the only other gases that might normally require consideration are carbon dioxide and ammonia. The solubility of these two gases is so great relative to oxygen and nitrogen that they can normally be ignored.

For the dissolved oxygen region defined by Equation 6 the oxygen content of the gas forced out of the system is the same as that in the aspirator unit, so that $J = X_1/p$. Then Equation 4 reduces to

$$\frac{dX_1}{dt} + \frac{K_1RT}{pv}(X_0 + Y_0)X_1 = \frac{K_1RT}{v}X_0 \tag{15}$$

The solution of Equation 15 is the following if for $t = 0$, $X_1 = X_2$; and for $t > 0$, the dissolved oxygen content of the water passing through the system is any arbitrary constant value in the region $X_0 > p - Y_0$:

$$\frac{X_1 - X_3}{X_2 - X_3} = \exp.\left[-\frac{K_1RT}{v}\left(\frac{X_0 + Y_0}{p}\right)t\right] \tag{16}$$

where

$$X_3 = \frac{X_0 p}{X_0 + Y_0} \tag{17}$$

Under these conditions, the partial pressure of oxygen changes from its initial value to its final steady value, X_3, exponentially with time according to Equation 16. A Henry's law equilibrium is never attained, so in this dissolved oxygen region the composition of the gas is influenced by many variables.

These relationships between the partial pressure of oxygen in the aspirator unit and the dissolved oxygen content of water and other variables are shown in Figures 2 and 3. The final partial pressure of oxygen in the aspirator unit (relative to the partial pressure of oxygen in water-saturated air at 1 atm. total pressure) is plotted as a function of the dissolved oxygen content of water in terms of per cent saturation.

In Figure 2, curve A, beginning at the origin and extending in a heavy straight line through points d, b, c, f, and e, is Henry's law. For the present system, the final partial pressure of oxygen in the aspirator unit conforms to Henry's law in a limited dissolved oxygen region only, as described by Equation 5, provided $J = 0$ and $K_3 \ll K_1RT$. The upper limit of this region, expressed in terms of per cent saturation, is given by

$$\frac{X_0}{X_4} = \frac{p - Y_0}{X_4} = \frac{P - W - Y_0}{X_4} \tag{18}$$

In Figure 2, the dissolved oxygen region for which the oxygen component of gas in the aspirator unit can conform to Henry's law extends from the origin to the dissolved oxygen content corresponding to any of the points b, c, d, e, and f. These points, in turn, depend on the total gas pressure, the vapor pressure of water at the temperature of the system, and the dissolved nitrogen content of the water passing through the unit, in accord with Equation 18. Table I summarizes the data used to calculate these points. The data are grouped into four pressure ranges, where pressure IIa is 20% greater than Ia. Because variations in barometric pressure affect the gas pressure of the system, pressure ranges Ib and IIb are included to illustrate the effect of a barometric pressure variation equivalent to 40 mm. of mercury that might be encountered under actual conditions. Pending different information, it is assumed that the dissolved nitrogen content of surface waters may normally vary approximately 10% from saturation.

Table I. Upper Limit of Henry's Law Region

	Pressure (P), Mm. Hg	p(20° C.), Mm. Hg	Y_0/Y_4, % Sat.	X_0/X_4, % Sat.	Figure 2 Point	Figure 2 Curve
	760	742.5	90	137.8	...	...
Ia	760	742.5	100	100.0	...	...
	760	742.5	110	62.2	b	B
	720	702.5	90	112.0	c	C
Ib	720	702.5	100	74.2	...	...
	720	702.5	110	36.2	d	D
	912	894.5	90	235.8	...	...
IIa	912	894.5	100	197.9	...	...
	912	894.5	110	160.1	e	E
	872	854.5	90	210.0	...	...
IIb	872	854.5	100	172.2	...	...
	872	854.5	110	134.3	f	F

Table II

L, % Saturation	K_1RT/v, Min.$^{-1}$	M, % Saturation per Min.
5.0	0.1	0.5
	0.5	2.5
	1.0	5.0
	2.0	10.0
	3.0	15.0

Pressure ranges I*a* and I*b* in Table I and points *b*, *c*, and *d* in Figure 2 show that relatively small changes in the dissolved nitrogen content of water and the barometric pressure can significantly influence the value of X_0/X_4, the upper limit of the dissolved oxygen range in which Henry's law behavior is observed for the oxygen component of the gas. The worst case that might be encountered under actual conditions limits this region to dissolved oxygen values less than 36.2% of saturation if the gas pressure in the system is at about 1 atm. This range, however, is expanded to 134.3% of saturation if the total gas pressure in the system is increased about 20%.

If the dissolved oxygen content of water is greater than the value defined by Equation 18, then the final partial pressure of oxygen in the aspirator unit deviates from Henry's law and depends on temperature, gas pressure, and dissolved nitrogen content of the water. This behavior is illustrated by curves *B*, *C*, *D*, *E*, and *F* in Figure 2. In this dissolved oxygen region, gas composition measurements are not sufficient to establish the dissolved oxygen content of the water. The use of the method is necessarily limited to the region in which Henry's law behavior can be observed within the limits of experimental error.

The effect of a finite gas entrainment rate on the final partial pressure of oxygen in the aspirator unit is illustrated in Figure 3, where Henry's law is the curve for which $K_3/K_1RT = 0.0$. Under actual conditions, gas entrainment cannot be completely eliminated; therefore, it is desirable to design the aspirator unit for maximum possible value of K_1 and minimum possible K_3, consistent with other operating factors, to minimize deviations from Henry's law.

Limitations. The finite response rate of the system is a performance characteristic that limits its scope of application. The time function described by Equation 12 is useful only in characterizing the response of an instrument to a "step" change in the dissolved oxygen content of the input water and has no direct significance relative to a gradual change in dissolved oxygen. In terms of projected use of the analyzer as a rapid survey instrument, it is of importance to establish the performance of the system when subjected to water with a variable dissolved oxygen content.

If the dissolved oxygen content of water varies at a finite rate at all times, then it can be shown from Equation 8 that $X_1 = X_0$ if the system responds instantaneously ($K_1 = \infty$) to any change in the dissolved oxygen content of water providing $J = 0$. For finite values of K_1, the actual partial pressure of oxygen in the system will lag behind the value it would have if the response were instantaneous. For the proposed applications, it is not necessary to know the actual lag ($X_1 - X_0$) as a function of time, but rather the maximum value of the lag under any condition.

It can be shown that at time of maximum lag

$$\left(\frac{X_1 - X_0}{X_4}\right)_{max.} = -\frac{v}{K_1RT}\frac{d}{dt}\left(\frac{X_0}{X_4}\right) \text{ if } J = 0 \text{ and } K_3 = 0. \quad (19)$$

Equation 19 was evaluated at that time at which the lag is a maximum, so that $\frac{d(X_0/X_4)}{dt}$ may be less than or possibly equal to the actual maximum rate of change in dissolved oxygen. In either case, if $M = \left(\frac{d(X_0/X_4)}{dt}\right)_{max.}$ and $L = [(X_1 - X_0)/X_4]_{max.}$ then

$$L \leqslant \frac{-M}{K_1RT/v} \quad (20)$$

That is, the maximum lag under any condition is always less than or equal to some multiple of the maximum rate of change of dissolved oxygen. In addition, the maximum lag, L, is a function of temperature, aeration rate and gas volume.

The implication of this is that although the system, if given sufficient time under the proper conditions, will conform to Henry's law for oxygen, there is a limitation in its application for practical situations that is associated with the rate at which the system responds. For any instrument that responds according to a specific value of K_1RT/v, if an arbitrary limit is set for L, then its suitability for use in a particular application is completely defined if the maximum expected rate of change of dissolved oxygen in water is less than that given by Equation 20.

In Table II, M is the greatest rate of change of dissolved oxygen in water for which an instrument that responds at a rate described by K_1RT/v is suitable for use if the maximum acceptable lag in gas composition is 5.0% of saturation. These values of M are far greater than the changes normally encountered in surface waters at any given location. Equation 20 and Table II, however, will dictate the maximum rate at which an instrument can be moved along a stream for rapid survey purposes so that the lag in gas composition will not be excessive.

Experimentally attainable values of K_1RT/v fall within the range of values presented in Table II. The value of K_1RT/v

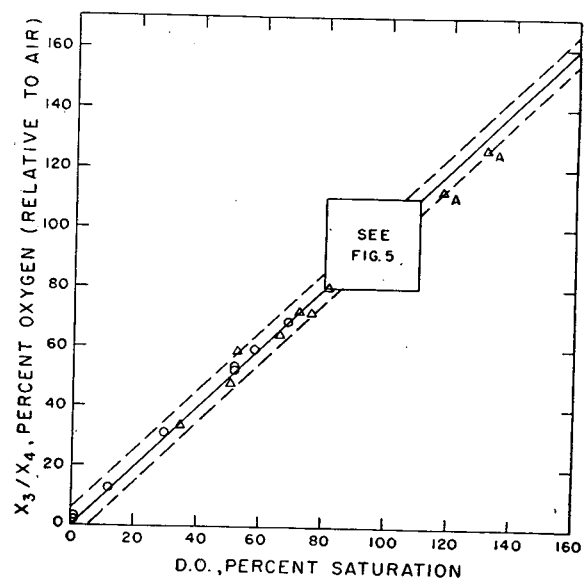

Figure 4. Oxygen content with M-1 dissolved oxygen analyzer at atmospheric pressure

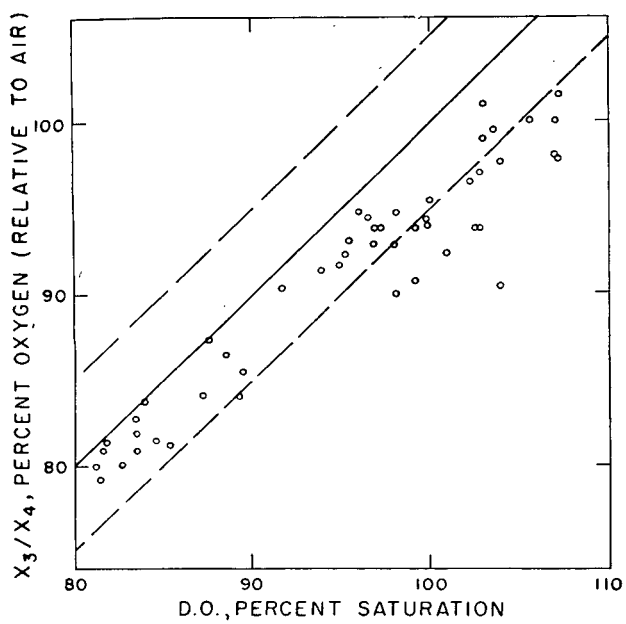

Figure 5. Oxygen content with M-1 dissolved oxygen analyzer at atmospheric pressure

Insert from Figure 4

can be increased by a decrease in the gas volume within the aspirator unit, which would minimize the gas composition lag. A very small gas volume, however, would magnify the pressure fluctuations caused in actual operation by rapid liquid level variations. Finally, to minimize the effects of physical gas entrainment it is necessary to maximize the value of K_1RT by increasing the number of gas bubbles and the water flow rate. Again, an increase in the flow of water requires a somewhat greater gas volume in the aspirator unit and is not consistent with satisfactory liquid level control. All these factors are closely interrelated, and a suitable design and performance compromise is difficult to establish.

EXPERIMENTAL

The water supply system consisted of two reservoirs and tap water connected in parallel. The dissolved oxygen content of the water in the reservoirs was adjusted by biological deoxygenation; by saturating hot tap water with air or nitrogen and cooling; by aeration with air, nitrogen, or oxygen; or by combinations of these procedures depending on circumstances. A mineral oil cover over the prepared water prevented reaeration.

Duplicate determinations of the dissolved oxygen content of the water supplied to the instrument were made by the azide modification of the Winkler method (*1*). These analyses were made at the same time any gas analysis measurement was made.

The aspirator unit of the first laboratory model analyzer (M-1) was similar in design to the typical system already described. A faucet aerator mounted on the end of the water inlet tube intimately mixed the oxygen-nitrogen mixture with the flowing water. A splash plate positioned beneath the faucet aerator minimized physical entrainment of the gas in the water leaving the system. A Type C Model 2 oxygen indicator (Mine Safety Appliances Co., Pittsburgh, Pa.) was used for gas analysis with a pump continuously cycling the gas from the aspirator unit through the gas analyzer and back to the aspirator again. Suitable valves and regulators were incorporated into this closed circuit to permit direct calibration and zeroing of the gas analyzer with air and nitrogen, respectively. A nitrogen supply was used to maintain the total pressure in the aspirator unit at $^3/_4$ inch of water above atmospheric pressure. In these experiments, the barometric pressure was not measured and the dissolved nitrogen content of the water was unknown. The water temperature was always measured at the outlet of the aspirator unit.

The results obtained with the M-1 instrument led to the construction of an M-2 analyzer with some improvements in design. The aspirator unit of the M-2 unit was a two-compartment cell forming a modified gravity flow aspirator (*3*). This arrangement permitted a reduction in gas volume to about 40 cc. and reduction in the flow of water passing through the unit to about $^1/_3$ gallon per minute. The aspirator unit also functioned as a gas pump and cycled gas through the gas analyzer and back to itself again. For gas analysis, a Gow-Mac Improved Standard differential thermal conductivity unit was used. The pressure in the aspirator unit was maintained at 1.2 atm. To accomplish this, a back-pressure diaphragm valve, adjustable from 0 to 24 inches of water, was used as a liquid level control with the gas pressure applied to both sides of the diaphragm to cancel out pressure variations.

It is estimated, from visual observation, that the entrainment rate was less than 0.5 cc. per minute for both instruments.

RESULTS

Response to Sudden Changes in Dissolved Oxygen Content of Water. A series of experiments was made with both dissolved oxygen analyzers to establish the final partial pressure of oxygen in the aspirator unit after sudden changes in the dissolved oxygen content of water from one constant value to another. The results are shown in Figures 4 to 6, in which the oxygen content of the gas in the aspirator unit relative to that of air at 1 atm. total pressure is presented as a function of the dissolved oxygen content of water in terms of per cent saturation. The results obtained with the M-1 analyzer for dissolved oxygen in tap water between 80 and 110% of saturation are presented in Figure 5 on an expanded scale. The temperature range covered by the series of experiments reported in Figures 4 and 5 is 7.2° to 36.3° C. For the experiments presented in Figure 6, the water temperature was maintained constant at 28.4° to 29.3° C., to eliminate interference of the signal of the thermal conductivity unit by changes in water vapor content. Points *A* in Figure 4 are experiments in which special precautions were taken to reduce the dissolved nitrogen content of the water so that deviations from a Henry's law behavior at higher dissolved oxygen contents would not occur.

The solid diagonal line in all three figures is the theoretical Henry's law response for the system and the dashed lines are a deviation of ±5% of saturation from the theoretical. With but one exception in Figure 4, all the results conform to Henry's law within ±5% of saturation. In Figure 5, however, the oxygen content of the gas in the system is consistently lower than the theoretical response, with the greatest deviation 13.6% of saturation. The discrepancy, on the whole, is greater with water of higher dissolved oxygen content. These results can be explained only in terms of barometric pressure variations or variations in the

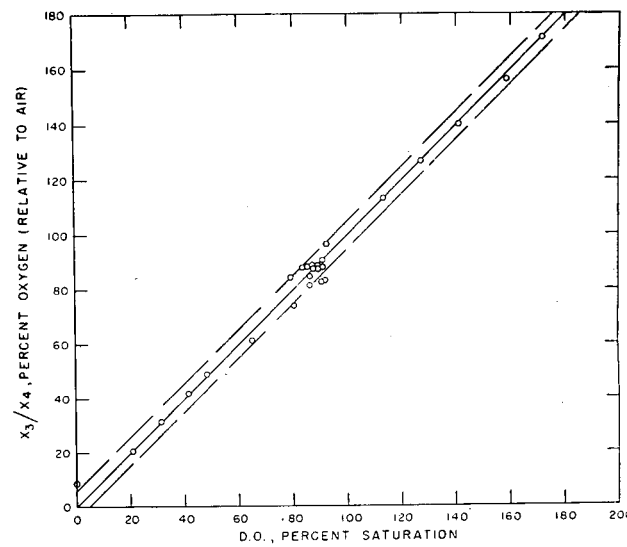

Figure 6. Oxygen content with M-2 dissolved oxygen analyzer at 1.2 atmospheric pressure

dissolved nitrogen content of the water. Within the limits of experimental error, all the results in Figure 6 conform to Henry's law up to 172% of saturation, with only four points deviating more than 5% of saturation from the theoretical. At this gas pressure, there is no apparent trend away from Henry's law that might be caused by the dissolved nitrogen content of the water or barometric pressure variations.

Response Rates. The rate at which the partial pressure of oxygen changed with time from its initial value to its final value for a step change in the dissolved oxygen content of water is illustrated in Figures 7 and 8 for experiments conducted with both instruments. In these figures, the fractional departure from the total partial pressure change for oxygen is plotted as a function of time on semilog paper. In such a plot, the response decreases from 1.0 as time increases from zero, irrespective of the initial and final dissolved oxygen contents of water and oxygen partial pressures of the system.

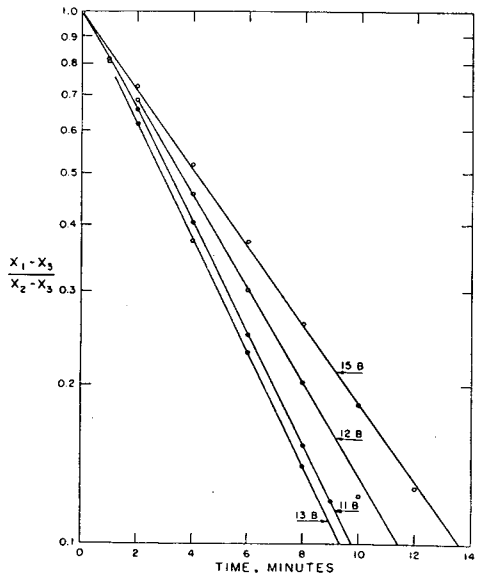

Figure 7. Response curves, M-1 dissolved oxygen analyzer

Typical results obtained with the M-1 instrument are illustrated in Figure 7 for experiments 11*B*, 12*B*, 13*B*, and 15*B*. For these experiments, the dissolved oxygen content of the water changed suddenly from about 100 to 0% saturation with the water temperature about 23° C. The gas volume was about 150 cc. for 11*B*, 12*B*, and 13*B* and about 215 cc. for 15*B*. Although the response was nearly exponential with time in accord with Equation 12, it was not possible to obtain good reproducibility in the response rate for replicate experiments. The value of K_1RT/v was found to be 0.24, 0.21, 0.25, and 0.17 min.$^{-1}$ for experiments 11*B*, 12*B*, 13*B*, and 15*B*, respectively. The corresponding values of K_1RT are 36, 32, 38, and 37 cc. per minute. If it is assumed that K_3 is 0.5 cc. per minute, then physical entrainment would cause approximately a 1.5% deviation from Henry's law. Such deviations are within the limits of experimental error for the entire dissolved oxygen content range reported in Figures 4 and 5.

Typical results obtained with the M-2 instrument are illustrated in Figure 8 for two experiments in which the dissolved oxygen content of the water suddenly changed from about 0% of saturation in one case and 172 in the second to 159 and 100% of saturation, respectively, at 29° C. The experimental response curves are not exponential with time. Good reproducibility of response curves in replicate experiments could not be obtained.

Contrary to the rapid response of the MSA gas-analyzer used in the M-1 instrument, the thermal conductivity unit did not respond rapidly to a change in gas composition. This response pattern for the thermal conductivity unit in the M-2 instrument is combined with that of the gravity flow aspirator unit. Curve *M*, shown in Figure 8 for comparison, was calculated assuming that the partial pressure of oxygen in the aspirator unit changed exponentially with time and that K_1RT/v = 0.51 min.$^{-1}$, where correction was made for the response rate of the gas analyzer. For all experiments conducted with the M-2 instrument, the value of K_1RT/v obtained in this manner was found to be on the order of 0.5 min.$^{-1}$. The corresponding value of K_1RT is about 20 cc. per minute and, if K_3 = 0.5 cc. per minute, physical entrainment would cause about a 2.5% deviation from Henry's law. Such deviations are within the limit of experimental error for the entire dissolved oxygen content range reported in Figure 6.

DISCUSSION

The design and performance characteristics of the major components of a prototype dissolved oxygen analyzer can be specified based on the experience gained with the two laboratory models.

As mentioned, a number of closely interrelated factors influence the design of the aspirator unit. For the two aspirator units employed in the present investigation, physical gas entrainment was found to have a negligible effect on the results under the conditions in which they were used. With the water flow rate at 1 gallon per minute, it was not possible to decrease the gas volume much below 150 cc. in the faucet aerator aspirator unit.

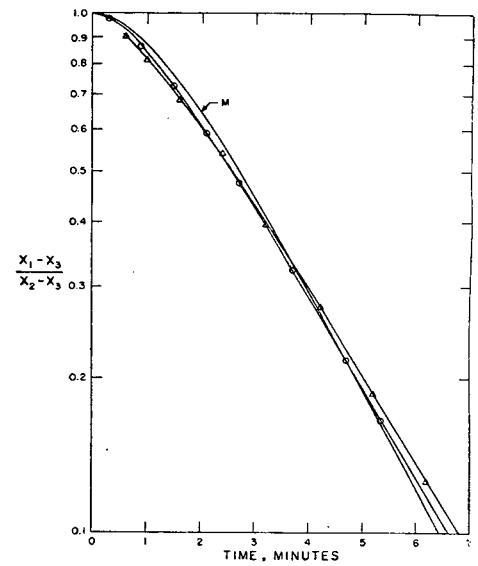

Figure 8. Response curves, M-2 dissolved oxygen analyzer

The 2.5-fold increase in the value of K_1RT/v for the gravity flow aspirator unit with a 40-cc. gas volume and 1/3-gallon per minute rate of water flow is advantageous in terms of a smaller gas composition lag and satisfactory liquid level control with a smaller gas volume. If necessary, the gas volume can be adjusted somewhat to obtain the best compromise between the value of K_1RT/v and pressure fluctuations caused by small but rapid variations in liquid level. Finally, the gravity flow aspirator unit has the additional advantage of functioning as a gas pump as well as a device for intimately mixing gas and water.

From these observations a gravity flow aspirator unit similar to that used in the M-2 instrument as illustrated in Figure 9 is

the more suitable design for use in a prototype dissolved oxygen analyzer for stream analysis.

Water enters the unit at point *A* at a constant rate, 1/3 gallon per minute, and overflows the gravity flow aspirator (5/16 inch in inside diameter) at *B*, entraining gas from the atmosphere above it in the process. The gas-water mixture falls through the tube into the lower compartment, striking splash plate *C*. The gas-water mixture separates, and gas is forced out of tubes *D* into the gas analyzer. The liquid level is controlled at a convenient point just below the splash plate *C*. Water drains from the system through *E*. Three perforated baffles, *F*, prevent water or foam from entering the gas analyzer. After passing through the gas analyzer, the gas completes its cycle by re-entering the unit at *G*. Nitrogen pressure is applied to the system at *H*. The two compartments are readily constructed from Lucite tubing about 1 3/8 inches in inside diameter, with the Lucite ends cemented to the tubes with dichloroethylene solvent. The entire unit must be gas-tight.

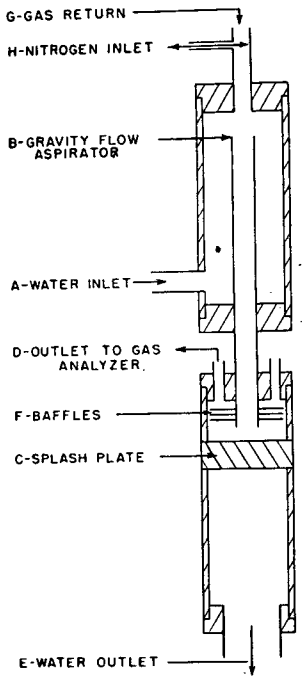

Figure 9. Aspirator unit

The essential components of a prototype model dissolved oxygen analyzer are arranged as illustrated in Figure 10. For satisfactory performance from such an instrument, the liquid level within the aspirator unit must be controlled without introducing large pressure fluctuations, so that surges of gas into or out of the system through the pressure regulator will not occur. Gradual changes in the liquid level relative to K_1RT/v can be tolerated within limits, because they would principally affect the response rate characteristics of the system. For best results, the control of liquid level should not be contingent on accurate regulation of input water flow rate. The back-pressure diaphragm valve used in the M-2 instrument performed well only with a well-regulated water input flow rate. It did not respond rapidly enough to accommodate the small variations in water flow rate that might occur in actual use.

Any gas analyzer specific for oxygen gas or any that can be made specific is suitable for use in the instrument, providing the analyzer will operate satisfactorily at 1.2-atm. total pressure and with pressure fluctuations on the order of 1 or 2 inches of water. In keeping with the objectives described earlier, the gas analyzer and the liquid level control should not be position sensitive and should not be affected by ambient temperature changes.

Two critical components of the instrument, the gas analyzer-recorder combination and the liquid level control, require further refinement. It appears that these problems can be solved in the course of normal development. None of the components of this instrument need to be large or heavy; thus, a truly mobile instrument can result from proper engineering development.

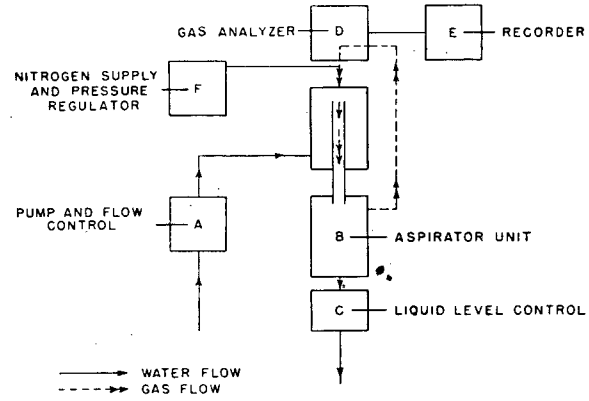

Figure 10. Schematic diagram of dissolved oxygen analyzer

The elements of a dissolved oxygen analyzer have been assembled and the successful functioning of the instrument has been demonstrated. However, the design of instrumental components has not been developed in final form. Private concerns may see an opportunity to use the information presented here for the final design of an instrument having important applications in many studies, particularly in water pollution investigations. The Robert A. Taft Sanitary Engineering Center plans to complete its work on development of this analyzer unless an instrument with acceptable performance characteristics is developed earlier by private industry.

ACKNOWLEDGMENT

The authors wish to thank the Mine Safety Appliances Co. for the loan of a Type C, Model 2 oxygen indicator.

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Colorimetric Method for Determination of Sugars and Related Substances

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Simple sugars, oligosaccharides, polysaccharides, and their derivatives, including the methyl ethers with free or potentially free reducing groups, give an orange-yellow color when treated with phenol and concentrated sulfuric acid. The reaction is sensitive and the color is stable. By use of this phenol–sulfuric acid reaction, a method has been developed to determine submicro amounts of sugars and related substances. In conjunction with paper partition chromatography the method is useful for the determination of the composition of polysaccharides and their methyl derivatives.

COLORIMETRIC tests for reducing sugars and polysaccharides have been known for a considerable time. The reagents such as 1-naphthol (*33*) for carbohydrates in general; benzidine for pentoses and uronic acids (*27, 49, 50*); naphthoresorcinol for uronic acids (*51*); and resorcinol (*43*), naphthoresorcinol (*39*), and resorcinol disulfonic acid (*31*) for ketoses are well-known examples of colorimetric tests that may be carried out in acid solution. Such tests as these and modifications of them using aromatic amines and phenols (*4, 22, 38*) have recently gained added importance since the extensive development of partition chromatography for the separation and characterization of minute amounts of sugars and their derivatives (*1, 4, 8, 11, 12, 17, 18, 21–23, 26, 36, 39, 47*). Polyols and carbohydrates with a reducing group may be detected by the Tollens silver reagent (*39, 52*), perhaps one of the best reagents in the art of chromatography. Reducing sugars are also detectable by picric acid (*7, 17*), 3,4-dinitrobenzoic acid (*5*), 3,5-dinitrosalicylic acid (*6, 32, 48*), *o*-dinitrobenzene (*17, 40*), and methylene blue (*54*), while diazouracil is said to be specific for sucrose as well as oligosaccharides and polysaccharides containing the sucrose residue (*42*).

Volumetric procedures involving the use of potassium ferricyanide (*19*), ceric sulfate (*45*), copper sulfate (*16, 44*), and sodium hypoiodite (*20*) are applicable to the determination of small amounts of reducing sugars after separation by partition chromatography. However, experience shows that these methods require considerable skill and are time-consuming and sensitive to slight variation in the conditions.

The anthrone (*13, 14, 28, 34, 35, 53*) and the 1-naphtholsulfonate (*10*) reagents are excellent for standard sugar solutions (*34*), but, when applied to the analysis of sugars separated by partition chromatography, the presence of only traces of residual solvent developer may render them useless. Most sugars can be separated on filter paper by a phenol-water solvent (*39*), but they cannot then be determined by the anthrone reagent because residual phenol, held tenaciously in the paper, interferes with the green color produced by the anthrone reagent. Moreover, the anthrone reagent is expensive and solutions of it in sulfuric acid are not stable (*30, 34*). The anthrone method also suffers from the disadvantage that, while it is satisfactory for free sugars and their glycosides, it is of limited use for methylated sugars and the pentoses. Although butanol–propionic acid–water is an excellent solvent for separating the disaccharides (*4*), the residual propionic acid interferes with the 1-naphtholsulfonate method. Aniline phthalate (*38*) and aniline trichloroacetate (*17*) have been utilized for the colorimetric determination of sugars and their derivatives (*2, 3*); these reagents, however, are unsatisfactory for ketoses.

Phenol in the presence of sulfuric acid can be used for the quantitative colorimetric microdetermination of sugars and their methyl derivatives, oligosaccharides, and polysaccharides (*15*). This method is particularly useful for the determination of small quantities of sugars separated by paper partition chromatography with the phenol-water solvent and also for those sugars separated with solvents which are volatile—e.g., butanol-ethanol-water (*39*), ethyl acetate–acetic acid–water (*26*), or methyl ethyl ketone–water (*4, 39*). The method is simple, rapid, and sensitive, and gives reproducible results. The reagent is inexpensive and stable, and a given solution requires only one standard curve for each sugar. The color produced is permanent and it is unnecessary to pay special attention to the control of the conditions.

DETERMINATION OF CONCENTRATION OF PURE SUGAR SOLUTIONS

Reagents and Apparatus. Sulfuric acid, reagent grade 95.5%, conforming to ACS specifications, specific gravity 1.84.

Phenol, 80% by weight, prepared by adding 20 grams of glass-distilled water to 80 grams of redistilled reagent grade phenol. This mixture forms a water-white liquid that is readily pipetted. Certain preparations have been known to remain water-white after a years' storage, while others turn a pale yellow in 3 or 4 months. The pale yellow color that sometimes develops does not interfere in the determination, inasmuch as a blank is included.

Coleman Junior, Evelyn, Klett-Summerson, or Beckman Model DU spectrophotometers. All were used with satisfactory results in this investigation.

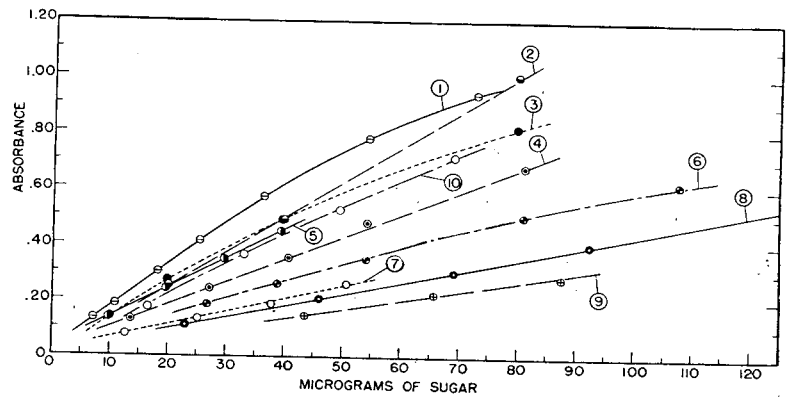

Figure 1. Standard curves

1. D-Xylose, Coleman Jr., 480 mμ, 17 mg. of phenol
2. D-Mannose, Beckman Model DU, 490 mμ, 40 mg. of phenol
3. D-Mannose, Evelyn, filter No. 490, 40 mg. of phenol
4. D-Galactose, Coleman Jr., 490 mμ, 33 mg. of phenol
5. L-Arabinose, Coleman Jr., 480 mμ, 17 mg. of phenol
6. D-Galacturonic acid, Coleman Jr., 485 mμ, 17 mg. of phenol
7. L-Fucose, Coleman Jr., 480 mμ, 40 mg. of phenol
8. D-Glucurone, Coleman Jr., 485 mμ, 17 mg. of phenol
9. 2,3,4,6-Tetra-*o*-methyl-D-glucose, Coleman Jr., 485 mμ, 17 mg. of phenol
10. D-Glucose, Beckman Model DU, 490 mμ, 100 mg. of phenol

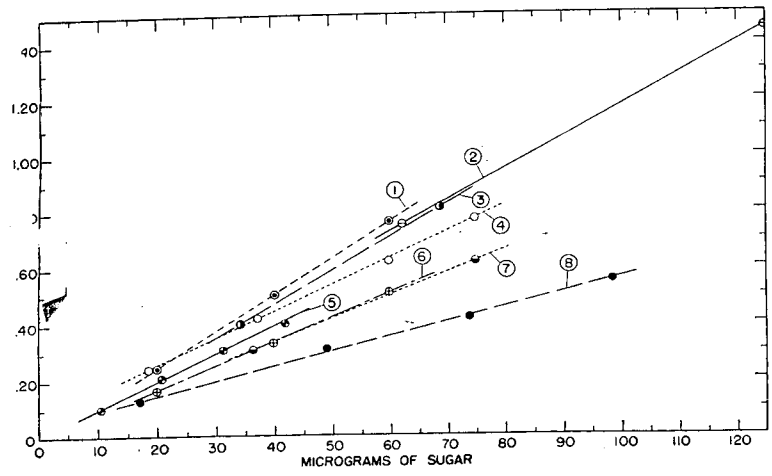

Figure 2. Standard curves

1. Sucrose, Beckman Model DU, 490 mμ, 100 mg. of phenol
2. Potato starch, Beckman Model DU, 490 mμ, 100 mg. of phenol
3. Dextran from *Leuconostoc mesenteroides* strain NRRL 512, Beckman Model DU, 490 mμ, 103 mg. of phenol
4. D-Glucose, Evelyn, filter No. 490, 80 mg. of phenol
5. L-Rhamnose, Coleman Jr., 480 mμ, 40 mg. of phenol
6. Raffinose, Beckman Model DU, 490 mμ, 100 mg. of phenol
7. D-Fructose, Beckman Model DU, 490 mμ, 200 mg. of phenol
8. 2-Deoxy-D-ribose, Coleman Jr., 490 mμ, 80 mg. of phenol

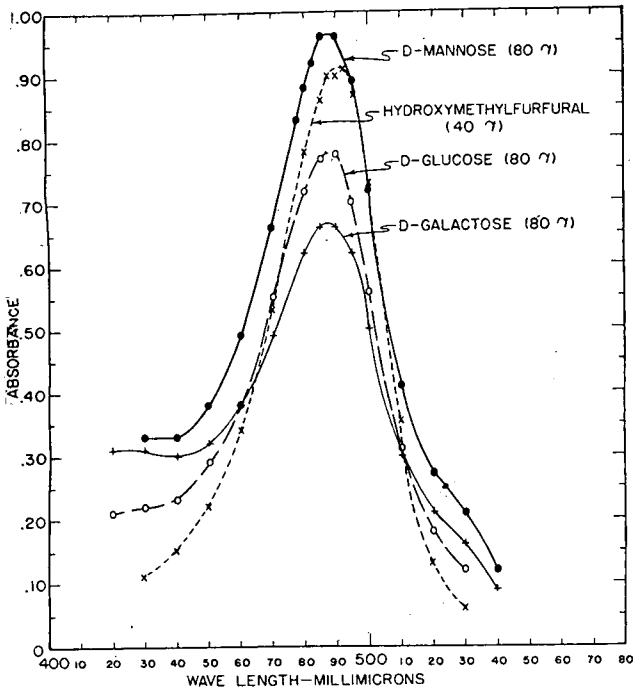

Figure 3. Absorption curves

Fast-delivery 5-ml. pipet, to deliver 5 ml. of concentrated sulfuric acid in 10 to 20 seconds. This is easily prepared by cutting a portion of the tip of a standard 5-ml. pipet.

Series of matched colorimetric tubes, internal diameter between 16 and 20 mm. This diameter will allow good mixing without dissipating the heat too rapidly. A high maximum temperature is desired because it increases the sensitivity of the reagent.

Series of micropipets delivering 0.02, 0.05, and 0.1 ml. The type described by Pregl (*41*) is satisfactory.

Procedure. Two milliliters of sugar solution containing between 10 and 70 γ of sugar is pipetted into a colorimetric tube, and 0.05 ml. of 80% phenol (adjust amount according to Figures 9 and 10) is added. Then 5 ml. of concentrated sulfuric acid is added rapidly, the stream of acid being directed against the liquid surface rather than against the side of the test tube in order to obtain good mixing. The tubes are allowed to stand 10 minutes, then they are shaken and placed for 10 to 20 minutes in a water bath at 25° to 30° C. before readings are taken. The color is stable for several hours and readings may be made later if necessary. The absorbance of the characteristic yellow-orange color is measured at 490 mμ for hexoses and 480 mμ for pentoses and uronic acids. Blanks are prepared by substituting distilled water for the sugar solution. The amount of sugar may then be determined by reference to a standard curve previously constructed for the particular sugar under examination.

All solutions are prepared in triplicate to minimize errors resulting from accidental contamination with cellulose lint.

If it is desired to avoid the use of micropipets, the phenol may be added as a 5% solution in water. The amounts of reactants are then: 1 or 2 ml. of sugar solution, 1 ml. of 5% phenol in water, and 5 ml. of concentrated sulfuric acid. All other steps are the same as above.

Standard Curves. A series of typical standard curves is shown in Figures 1 and 2. Included in these figures are examples of some of the sugars usually encountered in carbohydrate studies—namely, pentose, deoxypentose, methylpentose, aldohexose, ketohexose, hexuronic acid, disaccharide, trisaccharide, and certain methylated derivatives. In order to test the method, the experiments were repeated on different days and by different operators. In all cases the variations between experiments and between operators were no more than 0.01 to 0.02 unit in absorbance, which was the same order of magnitude as the variation between the triplicate samples.

The experimental data for the various carbohydrates, except 2-deoxyribose, given in Figures 1 and 2 may be tabulated by calculating the value of a_s, the absorbance index, in the equation $A_s = a_s bc$ (Table I). The absorbance, A_s, is a dimensionless ratio equal to $\log_{10} \frac{T_{\text{solvent}}}{T_{\text{solution}}}$, where T is per cent transmittance, b is the length of light path, expressed in centimeters, and c is the concentration, in micrograms of sugar per milliliter of final volume.

Discussion of Results. ABSORPTION CURVES. The curves obtained by plotting absorbance *vs.* wave length (Beckman Model

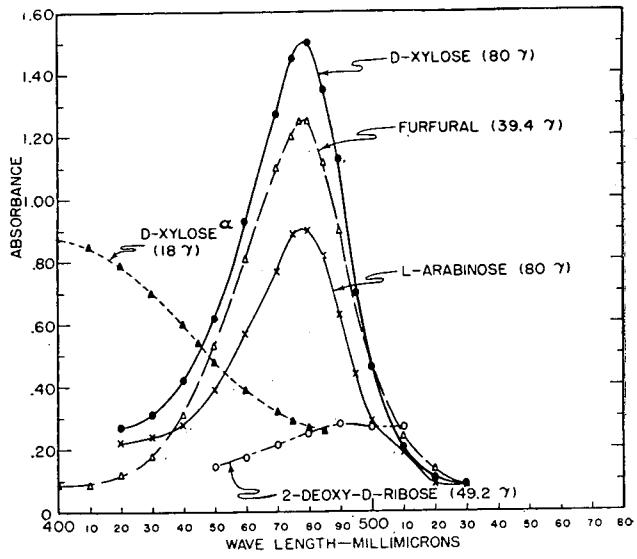

Figure 4. Absorption curves

A 0.1 ml. of butanol-ethanol-water chromatographic developing solvent (4 to 1 to 5, upper layer) was added in addition to the phenol

DU) are shown in Figures 3 to 8; the absorption curve is characteristic for each of the sugars described (*9*, *25*). The pentoses, methylpentoses, and uronic acids have an absorption maximum at 480 mμ, while hexoses and their methylated derivatives have an absorption maximum at 485 to 490 mμ. Certain of the methylated pentose sugars and their methyl glycosides show selective absorption at about 415 to 420 mμ (Figure 8) and for this reason the colorimetric determination of 2,3,5-tri-*o*-methyl-L-arabinose and its methyl glycoside is best carried out at 415 mμ.

The D-xylose and furfural curves are very similar. Assuming that the amount of color is proportional to the amount of furfural present or produced, the conversion of D-xylose to furfural under the conditions of the test is 93% of theory.

Calculation of conversion of D-xylose to furfural

	M.W.	Micrograms	Absorbance
Furfural	96	39.46	1.25
D-Xylose	150	80	1.50

The percentage, P, of xylose converted to furfural in the reaction as measured by the intensity of color developed can be calculated as illustrated below:

$$P = \frac{1.50}{1.25} \times \frac{39.46}{96} \times \frac{150}{80} \times 100 = 92.5\%$$

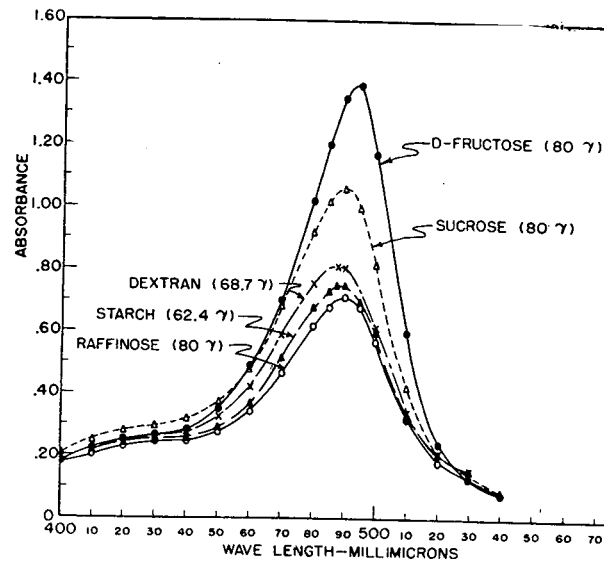

Figure 5. Absorption curves

Table I. Absorption Data for Certain Carbohydrates Determined by Phenol–Sulfuric Acid Reagent

Compound	Wt., γ	Phenol, Mg.[c]	Vol., Ml.	Light Path, Cm.	Instrument[a]	Wave Length, Mμ	Absorbance	a_s	Klett Reading
D-Fructose	37.4	40	6.60	1	B	490	0.31	0.0547	
	42.4	51.6	6.61	1	B	490	0.35	0.0545	
	42.4	103	6.64	1	B	490	0.48	0.0752	
	42.4	154	6.68	1	B	490	0.52	0.0819	
	42.4	206	6.72	1	B	490	0.47	0.0902	
	42.4	310	6.80	1	B	490	0.58	0.0928	
D-Glucose	42.2	51.6	6.61	1	B	485	0.45	0.0704	
	42.2	103	6.64	1	B	485	0.448	0.0702	
	42.2	154	6.68	1	B	485	0.40	0.0632	
	80	40	6.60	1	B	487	0.78	0.0640	
Sucrose	53.6	40	6.60	1	B	490	0.48	0.0591	
	26.3	40	6.60	1	B	490	0.237	0.0594	
	35	100	6.64	1.6	C	490	0.395	0.0468	
5-Hydroxymethyl-2-furaldehyde	40	154	6.68	1	B	490	0.86	0.143	
	40	206	6.72	1	B	490	0.95	0.159	
	40	257	6.76	1	B	490	0.98	0.166	
Starch	62.4	103	6.64	1.27	K	Blue, No. 42	0.0328	0.00275	16.4
	124.8	103	6.64	1.27	K	Blue, No. 42	0.064	0.00268	32
	187.2	103	6.64	1.27	K	Blue, No. 42	0.096	0.00268	48
	312.0	103	6.64	1.27	K	Blue, No. 42	0.146	0.00236	73
	62.4	103	6.64	1	B	488	0.75	0.0799	
	124.8	103	6.64	1	B	488	1.45	0.0772	
Dextran	34.36	103	6.64	1.27	K	Blue, No. 42	0.0166	0.00252	8.3
	68.72	103	6.64	1.27	K	Blue, No. 42	0.0338	0.00257	16.9
	137.44	103	6.64	1.27	K	Blue, No. 42	0.0646	0.00246	32.3
	286.4	103	6.64	1.27	K	Blue, No. 42	0.1380	0.00252	69
	34.36	103	6.64	1	B	488	0.40	0.0774	
	68.72	103	6.64	1	B	488	0.81	0.0784	
D-Galacturonic acid	80	16	6.58	1.00	B	480	0.532	0.0439	
D-Mannurone	80	40	6.60	1.00	B	485	0.39	0.0322	
D-Glucurone	80	40	6.60	1.00	B	480	0.287	0.0237	
D-Galactose	80.2	40	6.60	1.00	B	487	0.664	0.0546	
D-Mannose	80	40	6.60	1.00	B	487	1.01	0.0835	
L-Arabinose	80	40	6.60	1.00	B	480	0.90	0.0742	
D-Xylose	80	40	6.60	1.00	B	480	1.50	0.1239	
L-Rhamnose	80	16	6.58	1.00	B	480	0.82	0.0674	
L-Fucose	80	16	6.58	1.00	B	480	0.35	0.0288	
Maltose	40	100	6.63	1.6	C	490	0.47	0.0492	
Raffinose	50	100	6.63	1.6	C	490	0.46	0.0381	
Lactose	50	100	6.63	1.6	C	490	0.355	0.0294	
2-*o*-Methyl-D-xylose	50	20	7.45	1.6	C	485	0.31	0.0289	
2,3-Di-*o*-methyl-D-xylose	58.5	20	7.45	1.6	C	480	0.39	0.031	
Methyl 2,3-di-*o*-methyl-D-xyloside	47.7	35	7.45	1.00	B	480	0.23	0.036	
Methyl 2,3-di-*o*-methyl-D-xyloside	47.7	35	7.45	1.00	B	415	0.21	0.0328	
2,3,5-Tri-*o*-methyl-L-arabinose	40	50	7.45	1.6	C	415	0.27	0.0314	
Methyl 2,3,5-tri-*o*-methyl-L-arabinoside	50	50	7.45	1.6	C	415	0.325	0.0302	
2,3-Di-*o*-methyl-D-glucose	80	40	6.60	1.00	B	485	0.76	0.0708	
2,3,6-Tri-*o*-methyl-D-glucose	53	40	6.60	1.00	B	485	0.555	0.0690	
2,3,4,6-Tetra-*o*-methyl-D-glucose	80	120	6.65	1.00	B	485	0.57	0.0474	
2,3-Di-*o*-methyl-D-mannose	50	50	6.57	1.6	C	485	0.39	0.0320	
2,3,6-Tri-*o*-methyl-D-mannose	50	50	6.57	1.6	C	485	0.37	0.0304	
2,3,4,6-Tetra-*o*-methyl-D-galactose	50	50	6.57	1.6	C	485	0.37	0.0304	

[a] B, Beckman Model DU; C, Coleman Junior; K, Klett-Summerson.

[b] Klett reading $= \frac{1000 \times \text{absorbance}}{2}$

[c] Actual weight of phenol. To find weight of 80% solution, divide by 0.8.

Calculation of final volume

1 ml. water	2
1 ml. sulfuric acid × 1.84	9.20
Total wt.	11.20 grams

Concn. of sulfuric acid after mixing $\frac{9.20 \times 0.95}{11.20} = 78\%$

Density of 78% sulfuric acid (20° C.) 1.7043

Volume of mixture $\frac{11.20}{1.70} = 6.57$ ml.

The addition of small amounts of phenol was considered to have a negligible effect on the density of the solution; hence, 0.1 ml. of 80% phenol would increase the volume by 0.06 ml.

2 ml. water	2
1 ml. 5% phenol in water	1
1 ml. sulfuric acid	9.2
Total wt.	12.2 grams

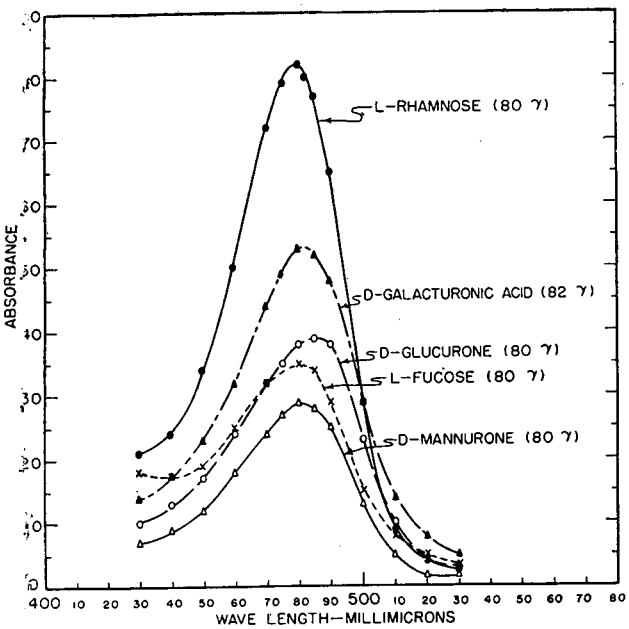

Figure 6. Absorption curves

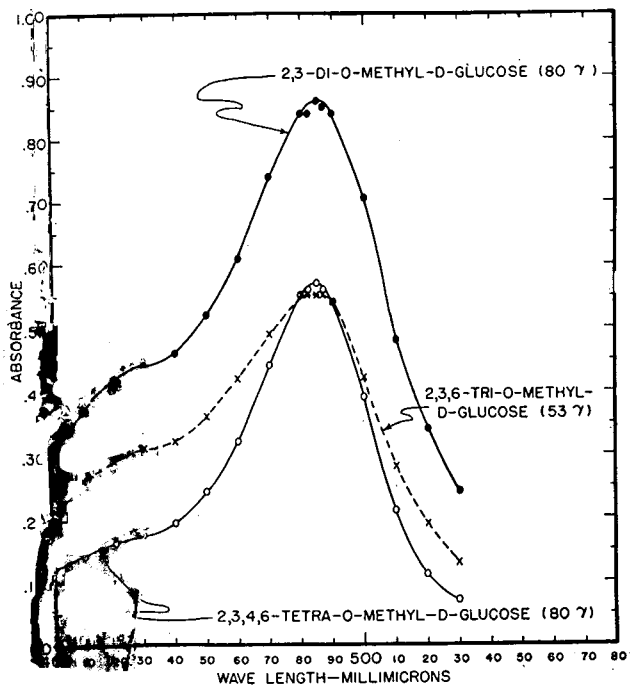

Figure 7. Absorption curves

Table II. Relationship between Index of Absorbance Sugar Concentration as Determined by Different Instruments

Instrument	Approx. Band Width, Mμ	D-Mannose, γ	Light Path, Cm.	Absorbance	a_s
Beckman Model DU	0.5	80	1.00	1.01	0.0835
	0.5	40	1.00	0.495	0.0815
	0.5	20	1.00	0.25	0.0826
Coleman Jr.	50	41.1	1.6	0.45	0.0451
	50	20.5	1.6	0.24	0.0481
	50	10.2	1.6	0.11	0.0442
Evelyn	65	40	1.9	0.49	0.0426
	65	20	1.9	0.27	0.0464
	65	10	1.9	0.13	0.0473

Concn. of sulfuric acid $\frac{9.20 \times 0.95}{12.2} = 71.6\%$

Density at 20° C. 1.628

Volume of mixture $\frac{12.20}{1.628} = 7.48$ ml.

Effect of Variable Amounts of Phenol. The intensity of the color is a function of the amount of phenol added. As the amount of phenol is increased, the absorbance increases to a maximum and then usually falls off (Figures 9 and 10). When a paper chromatographic separation has been effected using phenol as a solvent, it will be found impractical to remove all of the phenol developer by air drying. This is not essential, though, because the curve of absorbance *vs.* amount of phenol is relatively flat after the maximum color intensity has been reached. Reproducible results can be obtained by operating at either side of the peak or at the peak as long as the amount of phenol added is controlled. This could conceivably form the basis for the analysis of mixtures of sugars—for instance, of D-mannose and D-glucose—by making two series of experiments, one at low and one at high phenol concentrations. The difference in readings is not large enough by itself except for rather crude estimations, but in combination with the variation in wave length of absorption maxima peaks between pentoses or uronic acids and hexoses, a satisfactory analysis might be devised.

A procedure using a somewhat similar idea, the rate of color development between sugars and the anthrone reagent, has been reported by Koehler (*28*).

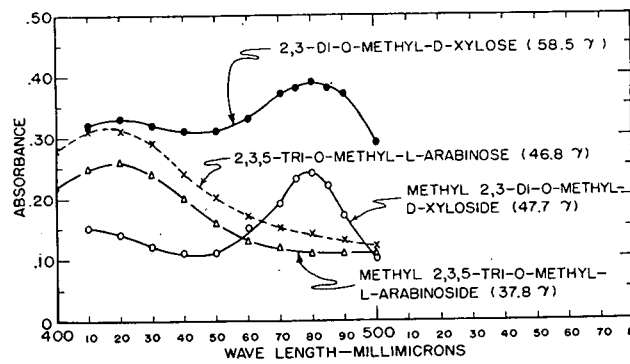

Figure 8. Absorption curves

Effect of Band Width. The absorbance, as is generally true in colorimetric determinations, is a function of the length of light path as well as the band width of the light source. As the band width becomes narrower, the observed absorbance becomes greater. If the values of the constant a_s are calculated from the equation $A_s = a_s bc$, the effect of the band width becomes apparent (Table II).

higher the value of a_s, the more sensitive is the instrument. [illegible]is basis, the Beckman was the most sensitive instrument used; the others, however, perform well enough for routine analysis.

In the case of the Evelyn and the Coleman colorimeters, the value of a_s is not constant. This means that the plot of concentration *vs.* absorbance is not linear at the higher concentrations; however, it is very nearly linear at lower concentrations. The linearity of the plot of absorbance *vs.* concentration is extended to higher regions of concentration by operating at narrower band widths. The points obtained in the nonlinear region with the colorimeters passing wider bands are, nevertheless, reproducible (Table II).

ACCURACY OF METHOD. Under the proper conditions, the method can be expected to be accurate to within ±2%. This figure was obtained by plotting the results obtained by use of the Beckman Model DU spectrophotometer and comparing the amount of sugar actually present with that indicated by the plot. As mentioned previously, the narrow band width of the Beckman spectrophotometer makes it possible to extend the linearity of the standard curve. The percentage error is shown in Table III.

Table III. Accuracy of Phenol–Sulfuric Acid Method for Sugar Determination

Compound	Taken, γ	Found, γ	Error, %	Absorbance
Mannose	80	81	1.3	1.01
	40	39	2.5	0.495
	20	20	0.0	0.25
Galactose	80.4	79.5	1.1	0.665
	40.2	39.5	1.7	0.325
	21.4	21.5	0.5	0.175

Conclusions. The phenol–sulfuric acid method can be used to give reliable estimations of the sugar content of pure solutions. The colors produced are unusually stable, and possess a definite absorption peak. The amount of color produced at a constant phenol concentration is proportional to the amount of sugar present. The standard curves obtained by plotting the sugar concentration *vs.* the absorbance can be readily reproduced and, because of this, only one standard curve need be prepared for a given sugar. Furthermore, the reagents are inexpensive, stable, and readily available.

QUANTITATIVE ANALYSIS OF SUGARS BY PAPER CHROMATOGRAPHY

The application of qualitative paper chromatography to the separation of sugar mixtures has been extended to the field of quantitative analysis. Any sugars that can be separated by the technique of paper chromatography can be determined quantitatively by the colorimetric technique just described after elution from the paper (*13, 15, 29*). The principle is simple, but certain factors complicate the analysis. Probably the most serious of these is that carbohydrate impurities are extracted from the paper along with the sugar to be analyzed. This source of error is reduced greatly by the simple expedient of running a blank. The size of the blank reading may be reduced to about one half by washing the papers with distilled water containing about 1% ammonia (*37*). Another complicating factor is the introduction of cellulose lint during the elution procedure, but this can be eliminated entirely by careful filtration.

A procedure similar to the one described herein is reported by Dimler and others (*13*). However, their elution procedure is considerably more complicated than the one used in this work. Furthermore, the best colorimetric technique at the disposal of these workers was the anthrone method, the disadvantages of which have already been explained.

Washing of Paper. The following experiment illustrates h[ow] the soluble carbohydrate fraction present in filter paper may [be] reduced by washing. This fraction cannot be entirely was[hed] out (*24*), and seems to increase after the washed paper is allo[wed] to dry (*46*). Other work (*24*) in this laboratory has shown t[hat] the soluble carbohydrate fraction of filter paper is of the na[ture] of a pentosan. The further study of this carbohydrate mate[rial] will form the subject of another communication.

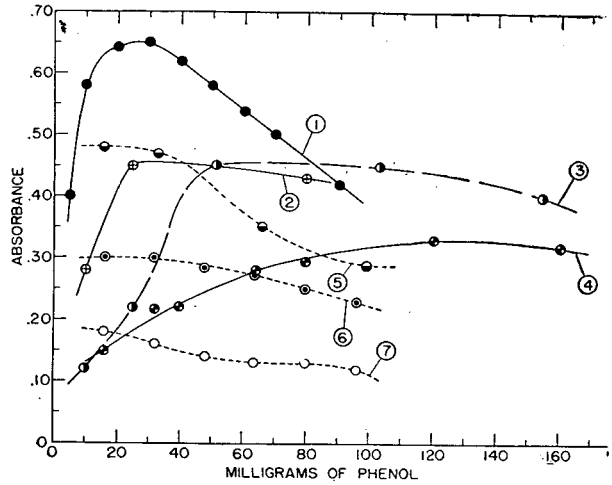

Figure 9. Absorbance *vs.* amount of phenol

1. D-Xylose, 40 γ, Coleman Jr., 480 mμ
2. D-Mannose, 41 γ, Evelyn, filter No. 490
3. D-Glucose, 42 γ, Beckman Model DU, 485 mμ
4. 2-Deoxy-D-ribose, 49 γ, Coleman Jr., 490 mμ
5. D-Galactose, 54 γ, Coleman Jr., 490 mμ
6. L-Rhamnose, 52 γ, Evelyn, filter No. 490
7. L-Fucose, 25 γ, Coleman Jr., 480 mμ

A piece of Whatman No. 1 filter paper 22 × 4 inches washed with distilled water containing 0.5% ammonia and for 24 hours. The paper was added to a beaker containing 2. of distilled water and allowed to stand with occasional shal for 20 minutes. The solution was filtered through a plug of g wool and a 2-ml. aliquot of it was transferred to a colorim tube. Forty milligrams of phenol was added as an 80% sol of phenol in water and then 5 ml. of concentrated sulfuric The absorbance of the solution was determined with an Ev colorimeter.

The solutions from the washed and unwashed papers sho[w] absorbances of 0.03 and 0.06, respectively.

Procedure. Two sheets of Whatman No. 1 filter paper 8 × inches are prepared as described below. One of the sheet[s] used as a blank. Before placing any sugars on the paper, li[nes] are drawn as follows: Two lines are drawn lengthwise 1.5 in[ches] from the edge of the paper. Two more lines are drawn, the[n] 1 inch from the top and the second 3.5 inches from the top. [The] sugars to be analyzed are placed on the paper along the 3.5-[inch] line. The two strips 1.5 inches from the edge are marking [strips] which will be cut off and sprayed with *p*-anisidine or *p*-phenet[idine] trichloroacetate or ammoniacal silver nitrate after develop[ment] of the chromatogram. The appearance of the spots ma[rks] distance the sugars have traveled in the marking strips [to] unsprayed center section. The amount of sugar added [to] marking strip is not critical as long as enough is present [to] a spot with the spray reagent. However, the amount of [sugar] added to the 5-inch center section of the paper must be accur[ately] measured if it is desired to determine the absolute amounts [of] sugars as well as the relative amounts in the mixture.

A margin of at least 0.5 inch should be allowed, leaving [5] inches in the center to which a measured amount of sugar solu[tion] can be added from a micropipet.

The amount of sugar which can be added before overlapping [of] the spots occurs should be determined for each type of analy[sis] This can be done by putting graded amounts of sugar on sep[arate] papers, drying, then developing with solvent, drying, and [then] spraying the entire paper. This will show whether the s[pots]

in discrete bands, and how much margin should be allowed the edges. The larger the amount of sugars which can be the less significance the blank will have. In most cases 1000 γ of sugar should be added. Dimler and others recommend that another paper be prepared to counteract variations in delivery that may occur with micropipets. To this paper add standard amounts of known sugars, using the micropipet and the same technique. This procedure does not eliminate the need for a blank determination, since the presence of the soluble carbohydrate fraction in the filter paper will have a relatively greater effect at low sugar concentrations. After the sugars have been added to the paper, the chromatograms are developed for a long enough period so that the sugars to be analyzed are clearly separated. After the chromatogram has been dried in the air, the side marking strips are cut off and sprayed to show the location of the sugars in the chromatogram. The center unsprayed portion of the chromatogram is then cut up into sections corresponding to the locations of the sugar. Each section is transferred to Petri dishes, beakers, or other suitable containers that can be covered or closed. The blank paper is cut up to correspond to the area and location of the sugars of the other paper. Twenty milliliters of distilled water is added to each of the Petri dishes, which are then covered and allowed to stand for 30 minutes with occasional shaking. During this time the sugar becomes equally distributed throughout the liquid and solid phases (water and cellulose). The eluate is filtered through glass wool and the concentration of sugars determined as described before, with the important difference that the absorbance of the blank reading is subtracted from that corresponding to the sugar before referring to the standard curve.

Results. Efficiency of Extraction of Sugars from Filter Paper. This is illustrated by two typical experiments:

1. With a micropipet, 0.102 ml. of a solution containing 4.52 mg. of D-fructose was added to a piece of Whatman No. 1 paper (3 × 5 inches). The paper was allowed to dry in the air for 24 hours and then soaked in 20 ml. of distilled water for 0.5 hour to extract the sugar. (In another series of experiments it was found that sugars are extracted from the paper almost immediately.) The extract was filtered through glass wool and a 2-ml. aliquot of the filtrate added to 20 ml. of water. Two milliliters of this diluted solution was treated with 258.4 μl. of 80% aqueous phenol, followed by 5 ml. of concentrated sulfuric acid. The observed absorbance at 490 mμ was 0.545 and 0.538.

In a blank experiment a piece of paper of identical size was extracted for 0.5 hour with 20 ml. of water. A 2-ml. aliquot was treated with phenol and sulfuric acid as described above. The absorbance was 0.10 (average of three results). Hence, the absorbance correction for the blank $= 0.10 \times \frac{2}{22} = 0.01$.

Corrected absorbance for the sugar determination $= 0.54 - 0.01 = 0.53$. From the standard curve for fructose, an absorbance of 0.57 is equivalent to 42.4 γ of sugar. Therefore, the amount of fructose equivalent to an absorbance of 0.53 $= \frac{0.53}{0.57} \times 42.4$ γ. Hence the total fructose recovered $= \frac{0.53}{0.57} \times 42.4 \times \frac{20}{21} \times \frac{22}{2} = 4336$ γ.

$$\text{Recovery} = \frac{4336}{4520} \times 100 = 96\%.$$

2. A similar experiment carried out with D-glucose (400 γ) added to a piece of paper (2 × 2 inches) gave a recovery of 100%. Additional experiments with D-mannose, D-xylose, and L-arabinose, and with methylated sugars such as 2,3,4,6-tetra-*o*-methyl-, 2,3,6-tri-*o*-methyl-, and 2,3-di-*o*-methyl-D-glucose with and without solvent migration using phenol-water, butanol-ethanol-water, and methyl ethyl ketone–water azeotrope gave recoveries of 95 to 100%.

Analysis of a Synthetic Mixture of Sugars. (1) A solution containing D-fructose (3.18 mg.) and D-glucose (0.20 mg.) was transferred to a piece of Whatman No. 1 paper (8 × 22 inches) as described previously. The chromatogram was developed for 24 hours by use of phenol saturated with water as the solvent. The paper was removed from the chromatographic chamber and allowed to dry for 24 hours. The marginal strips were cut off and sprayed with *p*-anisidine trichloroacetic acid reagent (small amounts of phenol do not interfere). After reassembling the chromatogram, the best line of demarcation was drawn between the two spots and the sections were cut out (glucose, 6 to 8.5 inches, fructose, 8.5 to 11 inches from the starting line), together with the corresponding blanks as previously described. The pieces of paper containing the two sugars and the two blanks were extracted and filtered. The concentration of the two sugars was then determined by the phenol–sulfuric acid reagent, reference being made to standard curves for glucose and fructose. The results were as follows:

Glucose Recovery

Absorbance of the eluate (2 ml. out of 20 ml. removed for test)	0.32
Absorbance of blank	0.10
Absorbance for glucose	0.22

From the standard curve for glucose absorbance, 0.45 = 42.4 γ glucose

Absorbance of 0.22 $= \frac{0.22}{0.45} \times 42.4$ γ glucose

Total glucose recovered $= \frac{0.22}{0.45} \times 42.4 \times \frac{20}{2} = 206$ γ glucose

Recovery = 103%.

Fructose Recovery

Absorbance of eluate (diluted 2 ml. to 20 ml. of water)	0.40
Absorbance of blank	0.01
Absorbance for fructose	0.39

From the standard curve for fructose absorbance, 0.57 = 42.4 γ fructose

Absorbance of 0.39 $= \frac{0.39}{0.57} \times 42.4$ γ fructose

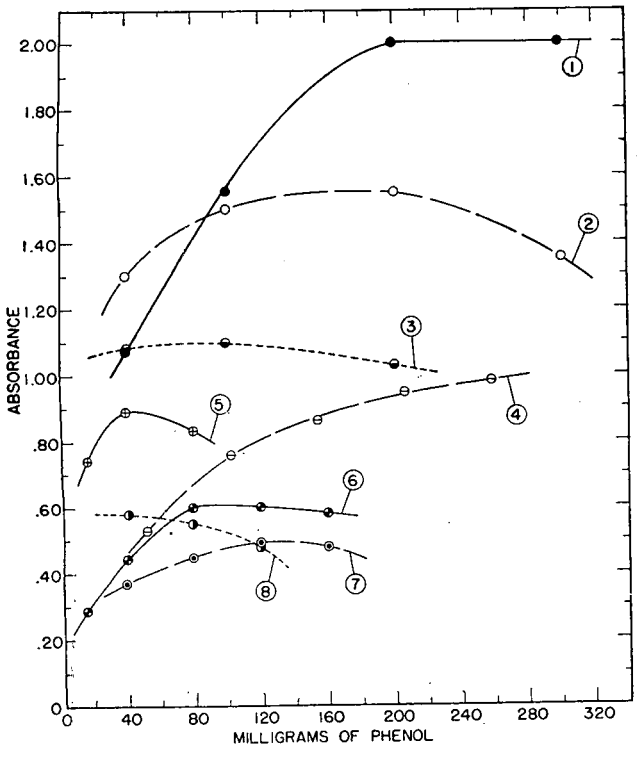

Figure 10. Absorbance *vs.* amount of phenol

1. D-Fructose, 80 γ, Beckman Model DU, 490 mμ
2. Sucrose, 80 γ, Beckman Model DU, 490 mμ
3. Raffinose, 80 γ, Beckman Model DU at 490 mμ
4. 5-(Hydroxymethyl)-2-furaldehyde, 40 γ, Beckman Model DU, 485 mμ
5. 2,3-Di-*o*-methyl-D-glucose, 80 γ, Evelyn, filter No. 490
6. 2-Furaldehyde, 20 γ, Evelyn, filter No. 490
7. 2,3,4,6-Tetra-*o*-methyl-D-glucose, 80 γ, Evelyn, filter No. 490
8. 2,3,6-Tri-*o*-methyl-D-glucose, 51 γ, Evelyn, filter No. 490

Total fructose recovered = $\frac{0.39}{0.57} \times 42.4 \times \frac{20}{2} \times \frac{22}{2} = 3200$ γ fructose

Recovery = 101%.

(2) For a solution containing D-mannose and D-glucose, the following results were obtained:

Solvent developer	1-butanol-ethanol-water
Time, hours	48
Paper	Whatman No. 3
D-Mannose added, γ	440
D-Mannose recovered, γ	417
% recovery	95
D-Glucose added, γ	470
D-Glucose recovered, γ	440
% recovery	93.5
Glucose in original mixture, %	51.5
Glucose calculated from analysis, %	51.3

The close agreement is fortuituous, but numerous experiments with mixtures of methylated and unmethylated sugars have shown that recoveries of 100 ± 5% or better are to be expected. In the above experiment the recoveries were not so good as expected, but it is believed that this is due to the fact that the sugar bands with Whatman No. 3 are less compact than those with Whatman No. 1; for this reason the No. 1 paper is preferred.

Table IV. Wave Length *Vs.* Absorbance for Starch[a]

(Starch-phenol-sulfuric acid, Beckman Model DU, slit width 0.1 mm., 103 mg. of phenol)

Wave Length	Absorbance for 62.4 γ Starch	Absorbance for 124.8 γ Starch
410	0.21	0.42
420	0.24	0.47
430	0.25	0.495
440	0.257	0.51
450	0.29	0.578
460	0.371	0.745
470	0.52	1.03
480	0.68	1.32
485	0.735	1.42
488	0.75	1.45
490	0.75	1.45
495	0.70	1.35
500	0.60	1.15
510	0.33	0.635
520	0.197	0.383
530	0.147	0.294

[a] Baker's potato starch dried for 3 days in vacuo (30 mm.) at 75° C.

Conclusions. The phenol–sulfuric acid method can be applied to the analysis of any mixtures of sugars and their methyl derivatives that are amenable to separation by paper chromatography. Thus it has been applied to the analysis of mixtures of methyl sugars separated on paper by butanol-ethanol-water or methyl ethyl ketone–water azeotrope. The method has also proved of value for the analysis of hydrolyzates of oligosaccharides; of polysaccharides such as starch (Table IV), glycogen, plant gums, and hemicelluloses (*15*); and for the determination of the amount of sugar in urine and in blood.

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Digester and Filter for Preparing Extract Solutions from Solids—Correction

After publication of the article on "Digester and Filter for Preparing Extract Solutions from Solids" [ANAL. CHEM. **27**, 1669 (1955)] attention was called to an article published a short time earlier by M. Potterat and H. Eschmann [*Mitt. Lebensm. Hyg.* **45**, 329–31 (1954)], in which a design for an apparatus having substantially the same features was presented. Since receiving this information the authors have sought to learn how the earlier article escaped notice and found that because of the time factor the publication in which it appeared could not have been available to them when the manuscript was prepared.

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Thermometric Determinations of Water and Acetic Anhydride in Acetic Acid

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The temperature rise occurring during the exothermic reaction of water and acetic anhydride in glacial acetic acid solution, catalyzed by a trace of perchloric acid, is employed to determine the concentration of either of these reactants in the presence of an excess of the other. Two different techniques are described. In one, the reaction is conducted in a Dewar flask. The temperature rise which takes place is a direct measure of the component being determined. By the other procedure either water or the anhydride is titrated in glacial acetic acid solution with a standard solution of the other until no further temperature rise occurs. Either procedure is conducted with adequate protection from atmospheric moisture. A qualitative method is described to detect the presence of water, acetic anhydride, or perchloric acid, within limits, in glacial acetic acid solution.

IN PREPARING the reagent for the partial acetylation of cotton (*6, 14*) in a pilot plant process, it was found that the heat evolved in the reaction between acetic anhydride and water is a measure of the reactant which is not in excess, thus providing a basis for its determination. It is evident that temperature changes will be greater for the same heat evolution in acetic acid solutions than in aqueous solutions, because of the difference in specific heat.

The heat of reaction has been proposed by Richmond and Eggleston (*21*) and Somiya (*28, 30, 31*) for determination of acetic anhydride in glacial acetic acid, and by Somiya (*27, 29*) for water in glacial acetic acid. These methods employ the heat of the reaction of the anhydride with aniline, but do not utilize catalysis by perchloric acid. Toennies and Elliott (*33*) use the perchloric acid catalyst in determining water in glacial acetic acid by a reaction with the anhydride, but measure an excess of that reagent by the optical rotation of its compound with *d*-camphoric acid. The rate of this reaction without catalysis has been studied by measuring physical constants (*1, 11, 32*) of the reaction mixture, or by determining the acetic acid formed (*26*), or the residual acetic anhydride (*5, 16, 17, 19*). The hydration without the catalyst is so slow that it does not interfere with accurate measurement of the catalyzed reaction by the heat evolved.

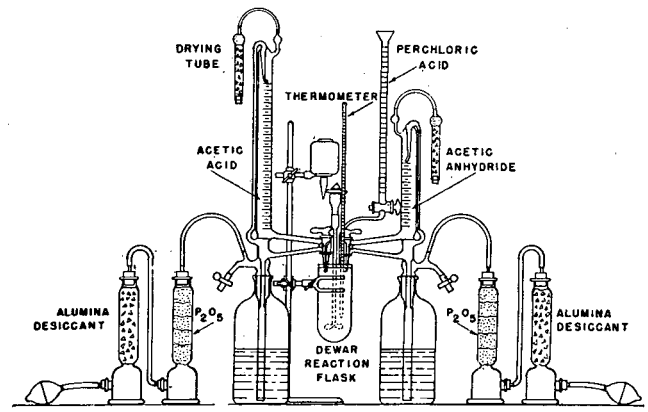

Figure 1. Test unit for heat of reaction of liquids

Comparisons of catalysts for the hydration of the anhydride (*7, 8, 14, 19, 22, 35*) have shown that perchloric acid is among the most powerful. The facility of control of that reaction made possible by that catalysis is the basis of the analytical methods proposed here.

Table I. Temperature Changes on Mixing Components of Acetylating Solutions

Step A. Acetic anhydride in varying proportions with glacial acetic acids of three different water contents, followed by
Step B. Addition of 0.1% by volume of 60% perchloric acid

Mixture, Vol. % AcOH	Step A. Temperature Drop, ° C., on Addition of Anhydride			Step B. Temperature Rise, ° C., (ΔT) on Addition of Perchloric Acid			$K = \frac{\Delta T - 0.90^b}{\text{Water Content}}$ for Mixtures with (AcOH)		
	A[c]	B[c]	C[c]	A	B	C	A	B	C
0	0.0	0.0	0.0	0.65	0.65	0.65	..	..	..
10.0	1.25	1.30	1.25	1.15	1.60	1.70	1.6	1.5	1.4
25.0	1.95	2.05	2.05	1.55	2.60	3.05	4.2	3.6	3.7
50.0	2.40	2.35	2.30	1.70	4.30	5.05	5.1	7.2	7.1
75.0	1.60	1.70	1.60	2.50	6.00	7.15	10.3	10.6	10.7
85.0	1.10	1.10	1.05	2.80	6.60	7.95	12.2	12.1	12.1
90.0	0.80	0.90	0.75	2.90	7.00	8.35	12.8	12.9	12.8
95.0	0.45	0.50	0.30	3.05	7.30	8.65	13.8	13.6	13.3
96.5		0.40	0.25	..	7.35	8.50[a]	..	13.7	..
97.5	0.20			3.00					
98.0			0.15			4.95[a]			
98.5		0.10			3.60[a]				
99.0	0.10			2.40[a]					
100.0				0	0	0			
				Av. ΔT per gram of water/100 ml.			14.2	14.4	14.2

[a] These points are at or to the right of the peaks in Figure 2, at which the increasing water and decreasing anhydride contents of the mixture became equivalent. The points of equivalence were computed to be 98.9, 97.2, and 96.7%, respectively, of the acids containing 0.156, 0.472, and 0.582 gram of water per 100 ml.

[b] This constant correction of 0.90° C. is for temperature rise due to water in the perchloric acid, and agrees with the average ΔT per gram of water per 100 ml. given above. The addition of 0.1 ml. of the 60.4% perchloric acid per 100 ml. of solution would introduce 0.0612 gram of water per 100 ml., to cause a rise of 0.88° C. based on 14.4° C. for 1 gram per 100 ml.

[c] Glacial acetic acids A, B, and C contain, respectively, 0.156, 0.472, and 0.582 gram of water per 100 ml. of acid.

BASIC CALORIMETRY

Apparatus. The calorimeter is shown in Figure 1. The Dewar flask was sealed with a cork stopper protected from the reagent fumes by aluminum foil. Any standard glass vessel holding about 100 ml. of the test solution in a depth to immerse the thermometer bulb fully may be employed.

Two thermometers were used, graduated respectively in 0.01° C. from 19° to 35° C. and in 0.01° C. from 0° to 100° C.

The particular stirrer used gave adequate mixing when turning a minimum of 600 r.p.m., and at that speed generated heat which caused a rise of 0.0035° C. per minute when immersed in 400 ml. of the usual reagent mixture. The thermometer employed agreed with a primary standard from

the National Bureau of Standards within 0.004° across any five degrees.

Exclusion of Moisture. Transfers of solution were conducted in air dried under pressure with aluminum hydroxide desiccant to contain less than 0.1 mg. of water vapor per liter of free air. Vessels were washed with reagent acetic acid which was rapidly evaporated from the walls with dry air.

Calorimetric Data for Acetylation Reaction. A series of mixtures with anhydride was prepared from each of three reagent acetic acids from the limits of 100% acid to 100% anhydride. A temperature drop occurred on mixing as shown in Table I and Figure 2, which was greatest at approximately the equimolecular proportion of 37.6% of the acid and 62.4% of the anhydride by volume. This effect was independent of the water contents of these acids. Reasonable search has not revealed previous report of it. Sandonnini (*23*) ascribed a temperature drop on adding water to glacial acetic acid to dissociation of complex molecules in both.

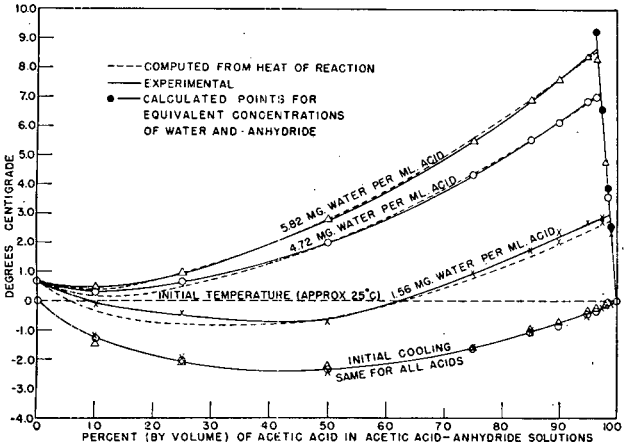

Figure 2. Temperature changes on mixing acetic acid with acetic anhydride

Before and after addition of perchloric acid

Immediately after the minimum temperature was reached, 0.1% by volume of 60% perchloric acid was added. A temperature rise occurred promptly in all mixtures (Figure 2). In mixtures of higher anhydride content, with the acid containing 1.52 mg. of water per milliliter, the temperature did not rise above that of the reagents before mixing. At times this behavior masked the observation of the temperature rise due to the hydration of anhydride during pilot plant studies of acetylation.

At the left of Figure 2, it can be seen that the addition of the perchloric acid to anhydride alone gave a rise of about 0.65° C. The rise computed from the heat of reaction of the water in this perchloric acid with anhydride in this system is 0.67° C., after correcting for the heat absorbed in dissociating the perchloric acid hydrate (*10*). However, except with pure anhydride, a correction of 0.90° C. accords with experimental evidence. The cooling due to dissociation of the perchloric acid hydrate may be compensated by heat from a reaction between perchloric and acetic acids. The increments of temperature increase above the initial cooling line, less 0.90° C., as given in the last column of Table I, are proportional to the extent of the hydration of anhydride. At the far right of Figure 2, each graph reaches a peak as decreasing content of anhydride and increasing content of water bring these to equivalence. Along the sharply descending line at the right of these peaks, water is in excess and the temperature rise in each case is in proportion to the anhydride content. Hence, descending lines are coincident for the three acetic acids.

The dashed lines along each graph in Figure 2 are those of computed temperature rise for the heat capacity of the liquid system. The heat of hydrolysis of acetic anhydride was taken as −13.96 kcal. per mole from Conn (*3*), specific heat at 20° C. of acetic acid as 0.487 (*9, 20, 24*), and of the anhydride as 0.434 (*13*). The temperature coefficient of specific heat for the acid (*9*) was assumed to be accurate also for the anhydride over the narrow range involved. The specific heat of perchloric acid was calculated by Kopp's rule (*15*). Heat capacities were computed for the temperature and composition after the reaction. Several assumptions were made in this preliminary study in the computations, both of theoretical rise values for Figure 2, and of the ratio K (Table I). First, losses to the calorimeter were neglected because the period of temperature rise, when anhydride was in excess, was less than that required for response of the thermometer. Also, changes in heat of the reaction due to the change in temperature during the reaction were neglected. It was recognized that these measurements were not of high accuracy. However, the magnitude of K, and its agreement over ranges of low water content common in acetylating reagents, predicted that a method of high precision and unusual accuracy for water in these solutions might be developed. It seemed probable that calibration curves would be required for each calorimeter and each proportion of anhydride to acetic acid employed.

QUALITATIVE TESTS

The presence of the three components—water, acetic anhydride, and perchloric acid—is required to give the temperature rise described above. This can be made the basis of qualitative tests for any of the three. The detection of water or anhydride is an important preliminary to the methods proposed later, particularly if the unknown solution contains both, which may continue to exist together for many days if no catalyst is present.

The tests which follow apply to solutions containing up to 50% anhydride but not more than 2% water, as used in partial acetylation of cotton, and containing no significant amounts of impurities which can be acetylated. This may comprise fresh reagents, mixtures of them, or spent solutions. In the latter case it may be presumed that nothing remains which can be acetylated further.

Procedure. A measured trace of perchloric acid is added to the unknown. If a temperature rise occurs promptly, the presence of the anhydride is indicated. If this rise exceeds that ascribable to the reaction with water in the perchloric acid, both water and anhydride were present. In that case the solution is cooled and divided into two portions. Addition to one of these of 10% by volume of anhydride will give a rise again if water was

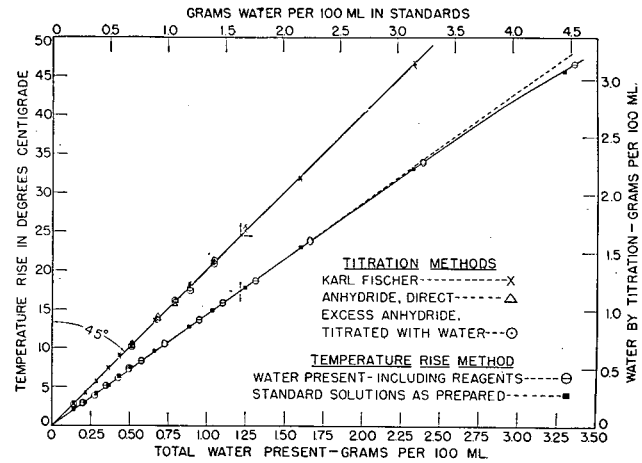

Figure 3. Thermometric determination of water in glacial acetic acid

present in excess of anhydride; addition to the other of 1% by volume of water will give a rise if anhydride was present in excess.

If no rise occurs on addition of perchloric acid to the original solution, 10% of anhydride is added. A rise, in excess of that ascribable to water in the catalyst, indicates that water alone was present.

If an initial rise occurs on addition of perchloric acid, but less than that ascribable to the water in it, the presence of anhydride less than equivalent to that water is indicated. To determine if a trace of water was also present, the solution should be tested with the water-free catalyst solution described later.

Perchloric acid is detected by adding slowly to the original solution 2% by volume of anhydride or water, whichever is found not to be present by the tests described above. A prompt temperature rise indicates the presence of perchloric acid, provided no other catalyst, which is generally a mineral acid, is present. The test is sensitive down to about 0.06% perchloric acid. If larger quantities of water or anhydride are added, the temperature drop when either is mixed with glacial acetic acid (Figure 2) may interfere with this test.

QUANTITATIVE PROCEDURES

Two procedures, applicable to the determination of water or of anhydride, were used. In one the temperature rise from the hydration reaction, when conducted in the calorimeter and catalyzed with perchloric acid, gives a direct measure of the reactant which is present in lesser proportion. The rise is inverse to the heat capacity of the system, so that calibration curves must be plotted for each combination of reagents and the calorimeter used. The speed of the catalyzed reaction reduces losses to the calorimeter flask.

In the second procedure, either water or anhydride is titrated by using the other as titrant in a glacial acetic acid solution containing sufficient perchloric acid to catalyze the reaction, with a sensitive thermometer to detect the rise of temperature. The end point is indicated by a cessation of temperature rise.

APPARATUS AND REAGENTS

Temperature Rise Method. Calorimeter (Figure 1).

Acetic anhydride, reagent grade, assay at least 98%.

Acetic acid, assay at least 99.5%.

Catalyst Solutions. Prepare a 1 to 10 dilution of reagent grade 60% perchloric acid in glacial acetic acid of at least 99.5% assay (catalyst A). Use of this solution necessitates a correction for water in the perchloric acid. Another catalyst may be prepared which will avoid this correction for water; it is referred to hereafter as "balanced catalyst." Prepare a 1 to 20 dilution of 60% perchloric acid in acetic acid with a calculated addition of anhydride to react with the water in both acids. Add the anhydride slowly while cooling the reacting mixture in ice water to below 30° C. Store the solution at about 5° C. in a frozen condition; a slight yellowing will occur, but no loss in catalytic power takes place within a week.

Titration to Cessation of Temperature Rise. Two mercury thermometers graduated respectively in 0.1° C. from 0° to 100° C., and in 0.02° C. from 18° to 34° C.

Erlenmeyer flask, 300-ml., with cork to fit. The cork is protected with aluminum foil, and has holes to admit the thermometer, buret tip, and drying tube connection.

Insulating jacket of $^1/_2$-inch wool felt for the Erlenmeyer flask.

Thermostat bath set at 2° C. above ambient temperature.

Balanced catalyst, described above.

2*M* standard solution of water in acetic acid.

2*M* standard solution of acetic anhydride in acetic acid.

If reagent grade acetic acid is used for either of these solutions, the water in it must be computed as present in the water solution, or as reducing the content of the anhydride solution.

Water-Free Acetic Acid. Acetic acid, essentially free of water or anhydride, is valuable for preparation of the standard solutions. It was made by the reaction of water in the reagent acid with a calculated addition of the anhydride. Lindemann (*16*) had attempted this, but lacked sufficiently accurate means of checking his product. The thermometric data and qualitative tests already given afford the necessary criteria.

The water content of the commercial acetic acid used is obtained by either Karl Fischer determination or the temperature rise method described later. The calculated equivalent of anhydride is added to the main solution, which is then heated to about 100° C. for several days, avoiding access of moisture. The product is tested for a residual excess of either water or anhydride by the temperature rise on adding the other. To test for water, 1% of anhydride is added, then 0.1% of 60% perchloric acid. A temperature rise above 0.90° C. from water in the catalyst will indicate water, in the proportion of 1 gram per 100 ml. for an increment of 14° C. (Table I). From the result, a second addition of anhydride to combine with a residual trace of water can be computed with high accuracy. With a thermometer graduated in 0.01° C., the sensitivity of the test will be better than 0.01 gram of water per liter. The use of balanced catalyst will avoid correction of 0.90° C.

An excess of anhydride should be avoided; but if present, it can be estimated from the temperature rise by adding 0.1% of 60% perchloric acid to the product of the first treatment, or more exactly by the direct titration method described later. Acid containing less than 0.05% of water can be prepared readily by these procedures. An apparent limit of accuracy of about 0.02% of water has been reached in handling this procedure.

DETERMINATION OF WATER

Thirteen solutions were prepared, containing from 0.19 to 4.44 grams of water per liter in reagent grade glacial acetic acid. Exposure to moisture was avoided. These standard samples served for preparation of a calibration curve for the temperature rise method, and as reference standards for the titrations. Water-free acid may be used for preparation of standards, but presence of anhydride in it must be avoided.

Table II. Determination of Water by Temperature Rise Method

Water in Standards as Prepared, Grams/100 Ml.	Water Present by Karl Fischer Analysis, Grams/100 Ml.	Av.	Temp. Rise Observed, ° C. (ΔT)	Av.	Temp. Rise, ° C., due to Water in Standards Only, $\Delta T - 0.90$[c]	Ratio of Corrected Rise to Water in Standards	Water Computed by Av. Ratio, Grams/100 Ml.
0.188[a]	0.185, 0.191	0.188	2.90, 3.00	2.95	2.05	10.90	0.190
0.287	0.281, 0.286	0.284	3.90, 3.95	3.925	3.03	10.56	0.281
0.387	0.379, 0.384	0.382	5.15, 5.05	5.10	4.20	10.85	0.389
0.486	0.500, 0.505	0.503	6.10, 6.10	6.10	5.20	10.70	0.482
0.585	0.599, 0.614	0.607	7.30, 7.35	7.325	6.43	10.99	0.596
0.687	0.715, 0.694	0.705	8.35, 8.30	8.325	7.43	10.82	0.690
0.886	0.916, 0.910	0.913	10.55, 10.50	10.525	9.63	10.87	0.894
1.183	1.21, 1.23	1.22	13.60, 13.55	13.575	12.68	10.72	1.178
1.383	1.41, 1.43	1.42	15.75, 15.80	15.775	14.88	10.76	1.382
1.662	1.69, 1.73	1.71	18.65, 18.65	18.65	17.75	10.68	1.648
2.15[b]	2.14, 2.15	2.14	24.00, 23.80	23.90	23.00[b]	10.70[b]	2.14
3.14[b]	3.12, 3.15	3.13	34.00, 34.10	34.05	33.15[b]	10.55[b]	
4.44[b]	4.43, 4.48	4.45	46.55, 46.55	46.55	45.65[b]	10.29[b]	
					Av.	10.785	

[a] Amount of water in glacial acetic acid before water dilution determined by Karl Fischer analysis.
[b] These were standards of high water content prepared to determine deviation from average of corrected rise–water ratio due to loss of heat to calorimeter. These ratios not included in computed average.
[c] The observed temperature rise is corrected in each case by 0.90° C. for water in catalyst A which was used in these determinations.

Temperature Rise Method. A 300-ml. sample and 100 ml. of acetic anhydride, both at about 1° C. above ambient temperature, are pipetted into the Dewar flask and mixed, and the lowest temperature reached is recorded. Then 4 ml. of catalyst A or 8 ml. of balanced catalyst is added. The temperature rise plotted against the concentration of water for each standard gives the calibration curve. Unknown samples are analyzed by the same procedure and their concentrations read directly from the curve.

The temperature rise line in Figure 3 is the calibration curve for this method, using the quantities of reagents employed in the present study with catalyst A.

Table III. Determination of Water by Thermometric Titrations

Water in Standards as Prepared, Grams/100 Ml.	Water Present from Temperature Rise Measurement (from Table II), Grams/100 Ml.	Water Present by Direct Titration with Anhydride, Grams/100 Ml.	Av.	Water Present by Excess Anhydride, Back-Titration with Water; Grams/100 Ml.	Av.
0.188	0.190	0.180		0.180, 0.182	0.181
0.687	0.690	0.711, 0.680	0.695	0.676, 0.692	0.684
0.908		0.945, 0.927	0.936	0.921, 0.921	0.921
1.059		1.045, 1.084	1.065	1.069, 1.084	1.076
1.183	1.178			1.192, 1.170	1.181
1.397				1.429, 1.401	1.415

Table IV. Determination of Water in Used Acetylating Solutions

Solution No.	Water as Prepared, Grams/100 Ml.	Temperature Rise, Obsd., ° C.	Temperature Rise Method, Grams/100 Ml.[b]	Karl Fischer Titration[a], Grams/100 Ml.	Ratio of Temperature Rise to Karl Fischer Titration	Back-Titration Using Excess of Anhydride[c] Grams/100 Ml.
1	0.35[a]	4.40	0.32	0.39	1.13	0.39
2	1.34	15.52	1.34	1.41	1.10	1.36
3	2.34	26.30	2.32	2.42	1.09	2.40
4	4.33	46.05	4.39	4.42	1.04	4.41

[a] To prepare this solution, the anhydride, present in a reagent mixture discharged from a pilot plant acetylation experiment, was determined by the temperature rise method. A calculated volume of water was then added to give a small excess above that equivalent to the anhydride; sufficient perchloric acid was added to catalyze the hydration reaction. Further measured additions of water to No. 1 gave Nos. 2, 3, and 4. Temperature rises were then determined by the addition of 100 ml. of anhydride to 300 ml. of the prepared solution and revising upward the observed rise by 1.67° C. to correct for the endothermic anhydride reaction. A small precipitate formed in these solutions; it was removed by filtration before the Karl Fischer and titration analyses. The slightly higher results obtained by these methods can be ascribed to water from the air introduced during these filtrations.

[b] These values were read from a calibration line plotted from temperature rise data given in Table II and shown in Figure 4.

Titration to Cessation of Temperature Rise. A 100-ml. sample of acetic acid under test is pipetted into a 300-ml. Erlenmeyer flask. Two milliliters of balanced catalyst is added, then 50 ml. of 2*M* acetic anhydride solution, with swirling and cooling to room temperature in the water bath to avoid overheating. The residual excess of anhydride is then titrated with 2*M* water solution. After each addition of titrant the flask is cooled in the water bath to within 2° to 3° C. above ambient temperature. Each 1-ml. addition of water solution causes a temperature rise of 0.3° to 0.6° C. As the end point is approached this rise becomes progressively slower. Additions of titrant are now reduced to 0.5 ml. and are continued until no further temperature rise is noted.

The 0.1° C. thermometer is used for the preliminary titration. In the final titration about 85% of the titrant may be added as fast as the solution can be cooled. Then the thermometer graduated to 0.02° C. is inserted and the titration completed slowly. When the end point is passed, a fall in temperature due to the endothermic reaction between water and acetic acid will occur. Because rapid cooling by the air may obscure the temperature increments as the end point is approached, the flask is removed from the bath, wiped dry, and placed in the insulating jacket. The use of the jacket accentuates the temperature rise from each addition of titrant and sharpens the end point.

When anhydride is present as well as water, the titration to cessation of temperature rise method gives only excess water above that equivalent to the anhydride, whereas the direct observation of temperature rise gives total water present.

This titration can be used for water concentrations up to 15 mg. per ml. of acetic acid. For higher water contents, the same excess of anhydride—i.e., about 20%—should be used; it may prove advisable to titrate less than 100 ml. of the unknown acid.

Direct titration with anhydride is not recommended because excessive time is required to complete hydrolysis.

Sample Calculation. Back-titration method for determining water in acetic acid: (50 ml. H_2O) × 0.03603 = grams of water present.

Comparison with Karl Fischer Method. Moisture determinations were made by direct titration with Karl Fischer reagent (*18*) to a visual end point in small Erlenmeyer flasks for the preparation of standard solutions and for comparative purposes (Tables II and IV).

DETERMINATION OF ACETIC ANHYDRIDE

Dilutions of anhydride suitable for the range of samples to be analyzed are prepared in water-free acetic acid. Eight solutions, shown in Table V, were prepared in this study. Standards may also be prepared with reagent acetic acid, in which case 0.1% of perchloric acid must be added to obtain immediate reaction between anhydride and the water in both acids. Then the anhydride content must be corrected for that used in reaction with such water, and the solution must be cooled to 25° C. before diluting to volume. The procedure given here is for standards prepared with water-free acetic acid without addition of catalyst.

Temperature Rise Method. A 200-ml. standard sample and 4 ml. of balanced catalyst are pipetted into the Dewar flask, and the temperature of the solution is recorded. Then 25 ml. of distilled water is added, slowly at first to avoid too much dilution of the catalyst, until a sharp temperature rise starts. The remaining water is run into the flask quickly and the maximum temperature reached is recorded. Temperature rise plotted against anhydride content by volume gives the calibration curve. Samples of unknown content are handled by the same procedure; the amount of anhydride present is read from the curve at the observed temperature differential.

The calibration line in Figure 4, from the data of Table V, involves a correction for the temperature drop, noted earlier (*23*) on mixing into acetic acid that part of water added which remained as an excess above the reaction with anhydride. This excess was computed for six concentrations of anhydride up to 65.5% by volume, equivalent to all of the 25 ml. of water, and the temperature drop for each was determined in a separate experiment. These values are plotted on a line below the horizontal axis in Figure 4. To the observed temperature rises, plotted to give the broken line, the amount of this drop at each point is added, which gives the corrected line shown. However, the uncorrected line is valid as a calibration curve if observed temperature rise is used directly to find the anhydride content. The addition of 25 ml. of water will allow a satisfactory excess up to 40% anhydride in 200 ml. of sample. However, for concentrations of 10% or lower it is advisable to use not more than 10 ml. of water and establish a new calibration line.

Titration to Cessation of Temperature Rise. To 100 ml. of the sample containing acetic anhydride in excess of any water present, is added 2 ml. of balanced catalyst. The solution is cooled to room temperature and titrated with 2*M* water solution. The technique of obtaining the end point is the same as that used in the titration of water.

As in the titration method for water, this procedure will determine only the excess anhydride if water is also present.

For accurate determination of anhydride content greater than 50% by volume, it is advisable to dilute the sample with water-free acetic acid and titrate a 100-ml. aliquot.

Sample Calculation. Milliliters of 2*M* water × 0.204 = grams of Ac_2O in sample.

DISCUSSION OF RESULTS

Applicability of these methods is indicated by the data in Tables II to V and Figures 3 and 4. The figures show typical calibration curves for both temperature rise methods.

The temperature rise data in Table II are plotted in Figure 3 against a horizontal coordinate (at the top of the graph) of concentrations of water in the standards, but across the bottom against concentrations in the calorimeter. The ratio of these coordinates is that of 300 ml. of the sample to 404 ml., when diluted with 100 ml. of the anhydride and 4 ml. of the catalyst. The temperature rise line, which is straight below 2.0 grams of

water per 100 ml. in standard solutions, is extended as a dashed line to higher values to show a deviation due to heat absorbed by the calorimeter.

The titration results from Table III, both Karl Fischer and thermometric, are plotted only against the water content of the standards, as shown by the horizontal coordinate across the top of this figure. The vertical coordinate on the right employed for the titration results is to the same scale, so that location of the plotted values on the 45° line shows agreement between preparation and titration of the standards.

The data of Table IV show that both the titration and temperature rise methods are valid for determination of water in used acetylating solutions. Test solutions were prepared by adding measured amounts of water to a stock solution, withdrawn from a step of a pilot plant experiment. This contained anhydride, for which a correction was made (footnote[a], Table IV). In comparing Tables II and IV it may be seen that the ratio of corrected temperature rise to the water present is very nearly the same.

Figure 4 shows the determination of acetic anhydride in acetic acid solutions by both techniques. The titration values coincide closely with the theoretical 45° line. Data from Table V of the temperature rise method applied to the same solutions give the broken curve before the correction, already discussed.

A few preliminary determinations of the anhydride content of used acetylating solutions showed good agreement between the titration and temperature rise methods.

The temperature rise procedure for water requires only a few minutes. The titration method requires about 20 minutes, which is longer than the Karl Fischer titration for water, but avoids the frequent preparation of the unstable Fischer reagent.

The temperature rise procedure for anhydride may require as long as 8 minutes when the excess of water is large. The time required for titration for anhydride is the same as for water. The aniline method (*17*, *25*) for anhydride requires 40 minutes for the initial saponification and 60 minutes for the acetanilide reaction and is no more accurate. Modifications of that method have used benzidine (*34*), 2,4-dichloroaniline (*2*, *4*), *m*-nitroaniline (*16*), or anthranilic acid (*12*) in place of aniline, but none of these appears to improve its speed.

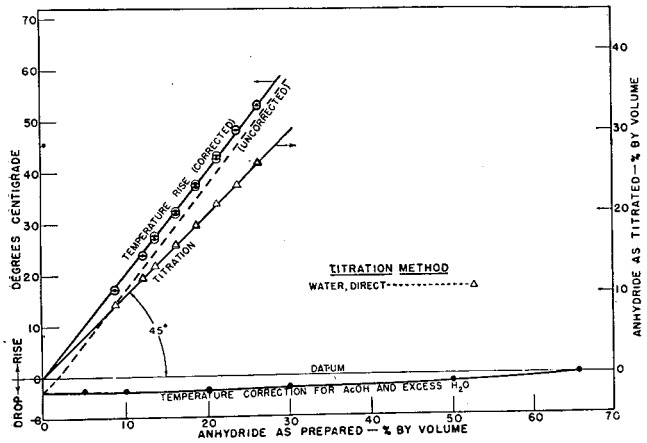

Figure 4. Thermometric determination of acetic anhydride in glacial acetic acid

Accurate and simple methods for determining water and acetic anhydride in glacial acetic acid have been tested in a narrow field of application to a single process. The temperature increases are generally in good agreement with those predictable from published values of the heat of the hydration reaction, without making corrections for heat absorption by the calorimeter or losses to the outside air. This agreement can only be ascribed to the speed of the reaction, and corrections will become necessary if the method is extended to conditions where the reaction is slower.

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Table V. Determination of Acetic Anhydride by Thermometric Methods

Acetic Anhydride by Volume as Prepared, %	Temperature Rise			Ratio of Corrected Temperature Rise to Anhydride Content of Mixture	Acetic Anhydride by Volume	
	Obsd.	Av.	Corr. for influence of excess water on acetic acid		By water titration, %	By temperature rise[a], %
8.76	14.2, 14.3	14.25	17.1	1.95	8.91	8.76
12.17	21.1, 20.9	21.0	23.8	1.96	12.15	12.00
13.67	24.25, 24.85	24.55	27.3	2.00	13.55	13.70
16.15	29.6, 29.2	29.4	32.15	1.99	16.20	16.03
18.62	35.1, 34.5	34.8	37.5	2.01	18.58	18.60
21.19	40.0, 40.6	40.3	42.95	2.03	21.15	21.26
23.57	45.7, 45.55	45.62	48.22	2.05	23.45	23.81
26.18	50.4, 50.7	50.55	53.05	2.03	26.10	26.18

[a] Values are computed from linear function derived from lowest and highest value, or essentially results which would be obtained by employing a straight line between these points as a calibration curve for the method.

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Platinum Oxide on Silicic Acid

Stable, Active Hydrogenation Catalyst

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Platinum oxide supported on silicic acid, a modification of the well-known Adams catalyst, is very active at room temperature yet is a very stable hydrogenation catalyst. The rate data obtained with the catalyst are reproducible and are of definite analytical value because they are dependent upon the chemical constitution of the material hydrogenated. In the determination of multiple bond unsaturation, its use is of advantage because it is conducive to short reaction times and sharp end points.

DURING the early stages of an investigation of the kinetics of catalytic hydrogenation undertaken previously in this laboratory, the main obstacle to satisfactory experimental work proved to be the instability of catalysts. This study concerned hydrogen absorption rates under normal conditions of temperature and pressure. The highly active catalysts needed for this type of work were prepared according to known procedures, most of them being obtained in the form of finely divided metals, such as platinum, palladium, or nickel, generally supported on some inert material and suspended in a solvent. A comprehensive review of the literature concerning this type of catalyst is now available (*3, 4*).

In spite of elaborate precautions involving cold storage in an oxygen-free atmosphere, the activity of the catalysts declined much too rapidly for comparable data to be secured. Further difficulties were encountered with catalysts such as Raney nickel, which could not be measured in precise amounts even by pipetting from thoroughly stirred and seemingly homogeneous suspensions. Platinum oxide prepared according to Adams (*1, 7*) was thought to offer a solution to this problem. It is easily prepared and can be weighed out accurately. On reduction with hydrogen, it readily provides a highly dispersed and very active form of platinum, which can be obtained *in situ* just prior to the introduction of unsaturated compounds. It is therefore not exposed to the effects of prolonged storage as is the case with most catalysts. Indeed, as determined from the behavior of equal amounts of the same platinum oxide preparation tested over a period of several weeks, only a slow over-all decline in activity was observed; the variation from run to run, however, was appreciable. It was obvious from the examination of samples which had displayed a particularly low activity that a change had occurred in the catalyst structure. The normally dispersed platinum microcrystallites had gathered to form agglomerates of variable size which settled rapidly when stirring was interrupted. The effect on the active area of this uncontrollable phenomenon is similar to that observed with other catalysts as a result of sintering through overheating.

It was thought that this tendency might be suppressed or at least minimized if platinum oxide was bound to a catalytically inert support. Martin (*6*) had described the preparation of supported platinum oxide catalysts for industrial use. These were not found active enough to warrant their use in this investigation.

A suitable catalyst could be prepared by including silicic acid in the preparation of the well-known Adams catalyst and by modifying the classical procedure to some extent. This new catalyst offers potential advantages to the analytical chemist. A few illustrative cases have been selected from the extensive data obtained through the use of the catalyst in a kinetic investigation to be reported at a later date.

All the data presented here resulted from experiments carried out at 25° C. and 760-mm. pressure, using the apparatus and techniques described earlier (*9*). One important feature of the apparatus is to allow control of the rate of hydrogen diffusion to the reaction medium by adjustment of the stirring. When stirring efficiency is increased beyond a certain point, hydrogen concentration in the medium remains constant—i.e., at the saturation value corresponding to the temperature and pressure used. Under these conditions, the variables affecting the rate of hydrogenation are the concentrations of unsaturated compounds initially involved, those of intermediate products, if any, and in some cases those of end products.

Application of the known simple tests to the absorption-time data invariably failed to reveal a simple relationship between rate and unsaturated compound concentration. This is in contrast to data obtained with catalysts such as Raney nickel (*9*), where a first-order relationship can be found. However, plotting the rate against the corresponding total unsaturation yielded characteristic curves which were used to establish the dependency of absorption rates on the variables listed above. In all experiments, the buret was read every minute and the rate at time t was taken as the average of the volumes absorbed at times $t - 1$ and $t + 1$. In spite of a small systematic error, this method proved to be more precise and more expeditious than the application to the time-absorption curves of the graphical tangent procedure. The total unsaturation at time t was taken as the corresponding volume of hydrogen still to be absorbed for complete reduction—i.e., the difference between the volume corresponding to complete saturation and that absorbed at the time considered.

PREPARATION

Seven grams of C.P. chloroplatinic acid are dissolved in 25 ml. of distilled water in a 100-ml. borosilicate glass beaker. Twenty grams of 200-mesh fraction No. 2847 silicic acid (Mallinckrodt) are added, and the whole is stirred with a glass rod until a smooth paste is obtained. The beaker, supported by a metal gauze, is placed over a burner and cautiously heated with continuous stirring until the mass is completely desiccated. The resulting powder is added by small portions with continuous and rapid stirring to 70 grams of molten C.P. sodium nitrate at 350° C. This is achieved by heating a 250-ml. beaker containing the nitrate with a strong flame, somewhat broader than the bottom of the beaker so that the walls are heated as well. Each addition of powder to the melt induces a temporary release of reddish nitrogen oxide fumes and a frothing of the mass. Additions are so spaced as to avoid any rising of the froth higher than about 1 inch above the original level. When all the powder has been added, any material which has solidified on the walls is melted, by use of an auxiliary heater, and is pushed down into the melt with the stirring rod. Heating and stirring are continued until gas evolution has practically ceased. The beaker is held with tongs while the contents are slowly poured into a 2-liter beaker containing 1.5 liters of distilled water, agitated by a mechanical stirrer. Another 20 grams of sodium nitrate are melted in the 250-ml. beaker. The melt is used to rinse the walls with the help of the glass rod, and then poured into the water. The beaker is allowed to cool, then it is washed with distilled water. The washings are added to the bulk of the preparation, which is stirred for 2 hours and allowed to settle overnight. As much clear supernate as possible is siphoned off and the residue equally distributed in centrifuge tubes. Water is added and the contents of each tube are well stirred with a glass rod. After centrifuging, the clear supernate is poured off and replaced by distilled water. The preceding operation is repeated

until the washings show just a faint pink coloration with phenolphthalein. The preparation is then transferred to a medium-porosity sintered-glass funnel with a minimum of water, and the liquid is filtered off. It is twice replaced by 95% alcohol, then ether. After being air-dried for 1 hour, the product is transferred to a vacuum desiccator containing phosphorus pentoxide.

The dried material is transferred to a small mortar, where it is easily pulverized, and then in small portions to a 200-mesh sieve. A camel's-hair brush is used to move the powder over the gauze. Any residue is reground and sieved until everything has passed through.

The light-brown powder is stored in a screw-capped bottle and well mixed before use.

The yield is 21.0 grams, containing 0.14 gram of platinum per gram of the material.

STABILITY

Microscopic examination of the reduced catalyst (PS 14) shows the platinum microcrystallites firmly embedded in the support. This markedly increases their stability. A sample of the catalyst, stored in a frequently uncorked bottle and exposed to full daylight for more than 2 years, has not sustained any appreciable loss of activity. Moreover, structural changes, such as previously observed with unsupported platinum, do not occur while the catalyst is being used. One immediate consequence of this high stability is the reproducibility of hydrogenation rate data, which is required for reliable analytical results.

ACTIVITY

Weight for weight, the platinum oxide in PS 14 is 2.5 times more active than the best Adams catalyst prepared in this laboratory, as judged by the time required for the complete saturation of a variety of olefinic compounds.

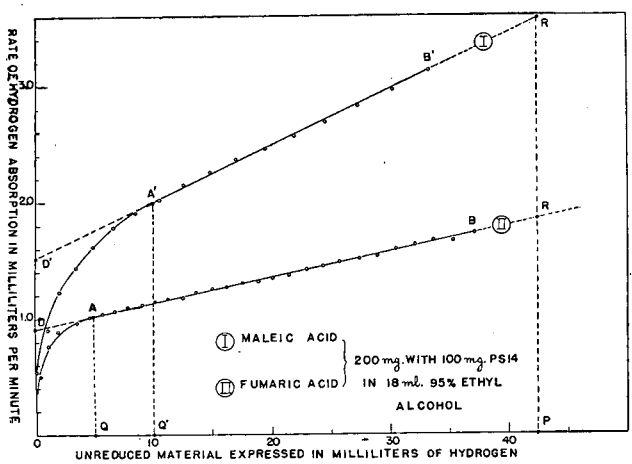

Figure 1. Hydrogenation rate curves of maleic and fumaric acids obtained under the same conditions

A comparison made with Raney nickel W-5 (*2*) in the reduction of long-chain unsaturated fatty esters shows PS 14 to be much more active. With only 20 mg. (3 mg. of platinum), the time required to reduce 256 mg. of methyl oleate was 11 minutes; with Raney nickel containing 191 mg. of nickel, the time to reduce 192 mg. of methyl oleate was 61 minutes. Half saturation of 254 mg. of methyl linoleate was attained in 5 minutes using 20 mg. of PS-14, while a sample of Raney nickel containing 200 mg. of nickel required 10 minutes to half saturate 192 mg. of the same ester. These data were selected from a series of experiments carried out in 95% ethyl alcohol.

Under these same conditions the benzene ring in phenol, benzoic acid, cinnamic acid, and benzamide is completely reduced in less than 2 hours, provided larger amounts of PS 14 are used.

Figure 1 shows the absorption rate curve when pure maleic acid (curve I) and pure fumaric acid (curve II) are hydrogenated in 95% ethyl alcohol. Both are typical of most curves obtained under these conditions. Curve II, for instance, displays two branches, OA and AB, distinct in character. Branch AB, which is a straight line, allows the initial rate PR to be obtained by extrapolation, as shown by the dotted lines. Thus PR is the ordinate corresponding to zero time for fumaric acid; PR' is the corresponding value for maleic acid. When these initial rates are plotted against amounts of catalyst used, straight lines passing through the origin are obtained, as shown in Figure 2. In the case of fumaric acid, somewhat higher values of the initial rates are obtained with higher initial concentrations of this acid; a 10% variation is observed when the amount of acid used varies between 50 and 200 mg. or when the volume of alcohol varies between 10 and 20 ml. With maleic acid only a 2% variation of the initial rate is observed within the same range of conditions. Thus, the initial rate corresponding to maleic or fumaric acid for one unit weight of catalyst affords a convenient way of defining the activity of a given sample.

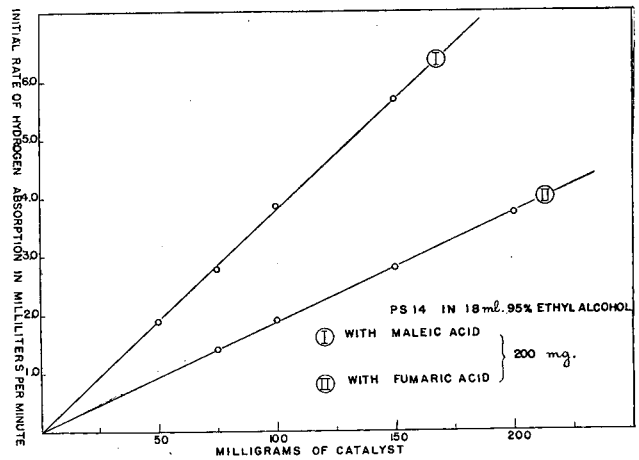

Figure 2. Amount of catalyst *vs.* initial rate plots for maleic and fumaric acids, showing simple proportionality

The advantage of maleic acid in yielding initial rate values practically independent of the initial acid concentration within relatively wide limits is offset, however, by the somewhat elaborate procedure (*5*) required to obtain the acid in a pure state. (The chemical which is sold as pure maleic acid is a mixture of the two stereo isomers.) Fumaric acid is stable, easily purified, and chemically well defined, and is thus to be preferred as a standard. As a measure of activity, called "fumaric acid value," the initial rate of absorption expressed in milliliters of hydrogen has been adopted. This is the value observed when 100 mg. of catalyst is used with 100 mg. of pure fumaric acid in 15 to 20 ml. of 95% ethyl alcohol under normal pressure at 25° C. The use of fumaric acid values should allow a direct comparison of results obtained in different laboratories. When the activities for the same unsaturated compound of two different samples of PS 14 (measured as initial rates obtained with equal amounts of the two samples) are determined, the ratio of these activities is usually found equal to the ratio of the corresponding fumaric acid values.

SELECTIVITY

Although the benzene ring in cinnamic acid is completely reduced, the reaction is much slower than the reduction of the side chain. A sharp break appears on the hydrogenation rate curve, clearly indicating the disappearance of the aliphatic double bonds; thus, it is possible to determine both aliphatic and aromatic unsaturation from the same experiment.

The benzene ring in phenol, benzoic acid, and benzamide is also reduced; it is left intact in phenol ethers and in alkyl benzoates. In aryl substituted alkenes and aryl substituted olefinic esters, only the side chain is reduced.

In 95% alcohol, PS 14 displays little selectivity in the reduction of polyunsaturated fatty acids and esters. If, however, the hydrogenation is carried out in dibutyl ether, there is a marked selectivity comparable to that observed with Raney nickel in 95% alcohol. This is exemplified in Figure 3, which shows three rate curves obtained with methyl linoleate. Curve I corresponds to PS 14 in 95% alcohol; it does not show the characteristic break indicating saturation of the 12-13 double bond observed in curves II and III, which were obtained with PS 14 in dibutyl ether and with Raney nickel in 95% ethyl alcohol, respectively.

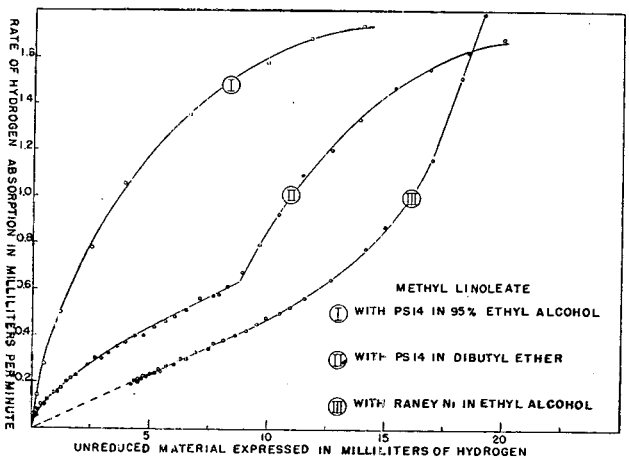

Figure 3. Hydrogenation rate curves obtained with methyl linoleate, showing differences in selectivity

Another type of selectivity is indicated by curves I and II in Figure 1. These were obtained with the same amounts of PS 14 and of the acids, in the same volume of 95% ethyl alcohol. Thus, maleic acid is reduced about twice as fast as its trans isomer.

Ethyl esters of these acids are reduced more slowly than the corresponding acids.

FACTORS INFLUENCING SELECTIVITY

The marked solvent effect upon selectivity described above for methyl linoleate is but one particular case of a more general phenomenon. The hydrogenation rate of any double bond is influenced by the reaction medium. For instance, while 20 mg. of PS 14 requires only 11 minutes to reduce 256 mg. of methyl oleate in 95% alcohol, 19 minutes are required in dibutyl ether to saturate 152 mg. of the same compound. This solvent effect may stem from many different causes. In this particular case it is due to the stronger adsorptive competition on the catalyst surface of the fully reduced compound. When increasing amounts of methyl stearate are deliberately added to identical mixtures of methyl oleate, dibutyl ether, and reduced catalyst, the observed initial hydrogenation rates are found to decrease with increasing amounts of the additive. Only a small decrease in rate is observed, on the other hand, when succinic acid is added to a reaction mixture containing either fumaric or maleic acid in 95% alcohol.

EFFECT OF INORGANIC ACIDS AND BASES

Attempts were made to speed up the removal of alkali from the freshly prepared unwashed catalyst, by neutralizing an aqueous suspension with inorganic acids to pH 7. This accelerated the final washing, but resulted in a very poor catalyst.

When very small amounts of sodium hydroxide are added to the 95% ethyl alcohol used as a solvent for the reduction of fumaric acid, an increase in activity is first observed. A maximum increase is reached for a 0.002*N* alkali concentration in the alcohol: There is a definite swelling of the support and an expansion in active area of about 100% as judged from the increase in hydrogenation rate. However, as the alkali concentration is further increased, the support gradually deteriorates and the activity declines. With a 0.05*N* solution, the catalyst no longer disperses normally throughout the reacting mixture, but forms a heavy flocculate having less than one fifth the activity of the normal catalyst.

Thus, both inorganic acids and bases affect the activity, apparently by inducing structural changes in the support. No satisfactory substitute for silicic acid has yet been found. Therefore the use of PS 14 should be avoided in strongly basic or acid reaction mixtures.

APPLICATIONS

Less than 20 minutes are usually needed to complete a quantitative determination of multiple bond unsaturation. Much larger amounts of other less active catalysts normally require 1 hour under the same conditions. This rapid and complete reaction is also characterized by a very sharp end point; there is no dragging of the absorption toward the end as with other catalysts. That this occurs with Raney nickel W-5 is shown in the following example: fifteen hours were required with 20.3 mg. of nickel (in the form of Raney nickel W-5) to reduce 250 mg. of methyl oleate in alcohol. The last 0.18 ml. of hydrogen was absorbed in just over 1 hour. When 10 times that amount of catalyst was used, the total time was reduced to 61 minutes, 0.24 ml. being absorbed in the last 10 minutes. In contrast with this, only 3 mg. of platinum (20 mg. of PS 14) were needed to hydrogenate 286 mg. of the same ester in 11 minutes, 0.51 ml. being absorbed in the last 2 minutes. On the basis of rate of hydrogen absorbed and weight of metal used, it would appear that the platinum in PS 14 is over 500 times more active than the nickel in Raney nickel.

Admittedly approximate, this figure indicates, however, a considerably higher activity at low concentration of unsaturated compound. Such a property is particularly desirable for hydrogen value determinations.

Comparative experiments carried out on a variety of compounds have shown it to be general in respect to double bonds under the conditions used. The superiority of platinum catalysts over nickel catalysts has been explained (*8*) on the basis of closer matching between atom spacing in the metal and carbon-carbon spacing of the unsaturated bond.

The extensive literature on batch-type catalytic hydrogenation under normal temperature and pressure includes an appreciable amount of hydrogenation rate data. Whether these data have any significance other than a qualitative one is doubtful. Most of them were obtained with catalysts, the activity of which could not be maintained constant long enough to allow these data to be duplicated. Furthermore, they were seldom recorded under all desirable conditions. By using an apparatus which allows such conditions to be established (*4*) it is possible to demonstrate that hydrogenation rate curves cannot be duplicated when these catalysts are used. Those obtained with PS 14, on the other hand, can be duplicated even after many months.

Such curves have interesting characteristics. Curve II in Figure 1 is typical of mono-olefinic compounds in ethyl alcohol. The initial rate *PR*, the coordinates of point *A*, the intercept *OD*, and the slope of line *AB* are all related to the particular type of double bond involved in the reaction. The position of this bond in the molecule; the presence, nature, and location of substituents; and the presence of activating functional groups influence the values of the above characteristics. For the initial rate alone

there is a wide range of values from zero (benzenic unsaturation, in some cases) to a very high one (allylic unsaturation).

With test materials containing different types of double bonds, the curve may, when the proper medium is used, show characteristic breaks such as in curves II and III, Figure 3. Such curves allow the quantitative estimation of the different double bonds by comparison with standard curves, which are established by using mixtures containing known amounts of the same constituents.

The full advantage of these properties may not be generally appreciated until a reasonably complete and systematic exploration of PS 14 behavior toward unsaturated compounds has been accomplished. Such a task requires access to a considerable number of pure, not commercially available, chemicals.

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Separation of Radioactive Silver-111 from Pile-Irradiated Palladium

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Radioactive silver-111, a 1-m.e.v. beta emitter with a 7.5-day half life, made by neutron irradiation of palladium metal, was separated in a remotely controlled, shielded, glass apparatus. The palladium metal was dissolved in aqua regia and 20 mg. of silver carrier as the chloride complex was added. The separation involved precipitation of silver chloride by dilution, dissolving again in ammonium hydroxide, followed by reprecipitation. The product was recovered in ammonium hydroxide solution or, if silver nitrate was desired, by metathesis to silver oxide, which was then dissolved in nitric acid. The yields were greater than 90%. The products contained less than 1 γ of palladium, and no activities other than that of silver-111 have been detected, even after decaying for 10 half lives. The silver-111 content was about 50 mc. in the highest activity product, and tests indicated that the method may be suitable for much larger quantities of silver-111.

PALLADIUM-110, of 0.135 natural abundance, is transformed by slow neutron capture (cross section about 0.4 barn) to radioactive palladium-111, which decays with a 22-minute half life by 2-m.e.v. beta emission to radioactive silver-111. The silver-111 is a 1-m.e.v. beta emitter with a 7.5-day half life, decaying to stable cadmium-111. Also, palladium-108 (natural abundance 0.268, slow neutron absorption cross section about 11 barns) forms radioactive palladium-109, a 1-m.e.v. beta emitter with 13-hour half life which decays to stable silver-109 (*3*).

Griess and Rogers (*1*) obtained carrier-free silver-111 by electrolysis from dilute palladium solution. Haymond and others (*2*) carried the silver-111 on a mercurous chloride precipitate, and later removed the carrier by evaporation at 450° C. Zimen (*8*) carried the silver-111 on a silver chloride precipitate, reduced the precipitate to silver using hydrogen at 500° C., and dissolved the silver in nitric acid. Rouser and Hahn (*4*) reduced the ammonia complex of the silver-111 and carrier silver in the palladium solution with vitamin C, and dissolved the metallic silver in nitric acid. Schweitzer and Nehls (*5*) utilized a method based on the formation of radiocolloids in basic solution, and Sunderman and Meinke (*7*) exchanged the silver-111 from a solution with the silver of a silver chloride film on platinum gauze.

In the work reported here, 1-gram foils of palladium metal, irradiated in an Oak Ridge National Laboratory reactor for a week, were allowed to "cool" for 4 days before processing, after which time the more intense palladium-109 activity had decreased to about the same level as that of the silver-111.

The separation was performed by dissolving the palladium foil in aqua regia, adding inactive silver carrier, then concentrated hydrochloric acid to assure complete solution of all the silver as its complex chloride ion. Silver chloride was precipitated by dilution with water, then dissolved in ammonium hydroxide and reprecipitated for further purification. The silver chloride was dissolved in ammonium hydroxide for the final product. However, if silver nitrate was desired as the final product, sodium hydroxide was added to precipitate silver oxide, which was then dissolved in nitric acid.

The silver chloride from the first precipitation always showed the same slight tan discoloration, indicating palladium contamination. However, the amount of palladium adsorbed on the precipitate was clearly shown to be less than 10 γ by dissolving one of the precipitates in 4 ml. of concentrated hydrochloric acid and comparing the color of the solution and its absorption (at the 460-mμ absorption band of palladium–hydrochloric acid solutions) with similar solutions containing known amounts of palladium. The palladium decontamination factor for a single precipitation is about 10^6; therefore, the palladium contamination after reprecipitation should be only a very small fraction of a microgram. None of the reprecipitated silver chloride products showed any tan discoloration, and a concentrated hydrochloric acid solution of one showed no absorbancy at 460 mμ, under such conditions that the limit of detection was about 1 γ of palladium. The color of the silver chloride precipitate is therefore a very delicate indication of the palladium contamination—e.g., if the precipitate is white, the palladium content is surely less than a few micrograms.

No foreign radioactivities have been detected in the products. Gamma spectra were recorded automatically with a sodium iodide (Tl) crystal spectrometer, and the spectrum was not changed by further purification. The activity of a product has been followed for 10 half lives, with no departure from linearity in the log activity *vs.* time curve.

The separation procedure was followed using unirradiated palladium and silver carrier containing purified silver-110 (270-day half life) with an activity of about 20 mr. per hour at 10 cm. The activity of all solutions was measured with a Geiger-Müller survey meter, and also by aliquot counting. The yield was greater than 90% in both cases. The yield was also

determined from an aliquot of the product by weighing, showing 93% recovery of the silver initially added.

Each 1-gram foil irradiated in the graphite reactor gave about 2 mc. of separated silver-111. Two grams of palladium, irradiated in the low intensity test reactor, gave about 50 mc. of silver-111. Separations were made from as much as 5 grams of palladium without any difficulty, which indicates that this procedure should be suitable for at least 0.1-curie, or possibly multicurie quantities of silver-111.

APPARATUS

The separations were carried out in glass equipment so connected that all necessary operations and transfers could be carried out by regulating pressures in the appropriate vessels with a laboratory rotatory pressure and vacuum pump. The glass vessels were mounted in a hood behind adequate shielding, and over a pan of sufficient volume to hold all the liquids used, in case of breakage. The manifold of stopcocks and the pump were outside the shielding. Mirrors and lights were placed over and inside the shielding, so that all operations could be controlled visually.

The more important details of the equipment are shown in Figure 1. Similarly numbered openings in the glass vessels are connected together through the corresponding stopcocks with $^3/_{16}$-inch Tygon tubing. Vessel *A* is a 2-liter Stang-type solution and precipitation vessel (*6*) with steep sides to minimize precipitate holdup on the walls. A medium-porosity borosilicate fritted disk, 30 mm. in diameter, is sealed in the bottom of *A*. Below the filter is an electromagnetically operated funnel, which allows the waste filtrates and washings to be drawn into the 3-liter filtrate receiver, *B*, and the product solutions to be withdrawn through the product funnel. The stopcock on the product line was operated remotely with a rod through a hole in the shield. Each solution in vessel *B* could be transferred to the 3-liter temporary storage chamber, *C*, from which it could then be returned to vessel *A* for reprocessing, if desired, or to a more permanent shielded waste container through tube and stopcock *E*. The 1-liter trap, *D*, prevents spray carry-over from going into the sulfuric acid and Ascarite traps used to protect the pump. All joints and stopcocks were fastened with spring clamps, and a mercury manometer pressure relief valve was used, set to relieve air from the system at 18 cm. of mercury.

Reagents were added remotely to reaction vessel *A* through a tube that could be directed by long tongs. An infrared lamp was focused on vessel *A* for heating its contents. Liquids could be held or mixed above the fritted disk for any desired length of time by the application of pressure in *B*. The apparatus was airtight, so that the turning off of all stopcocks connected to *B* would retain liquid in *A*.

PROCEDURE

Each process step was developed and practiced with inactive and tracer materials until it was shown to be reliable, and until a thorough familiarity was obtained with each manipulation, including appropriate pressure or vacuum settings on the pump, time and temperature relations, and the like. Complete tracer and "hot" runs were then carried out with careful monitoring, increasing the ratios of irradiated to inactive palladium, to develop appropriate modifications in equipment, shielding, process, and manipulation. The final procedure follows.

About 8 ml. of aqua regia are placed in vessel *A*, and the irradiated palladium foil is then added with tongs. Heat is applied with the infrared lamp until the palladium is dissolved. This will require less than 45 minutes if the solution is heated above 65° C. Evaporation to dryness should be avoided to prevent formation of difficultly soluble oxides. Five milliliters of concentrated hydrochloric acid are added, followed by the inactive silver carrier in hydrochloric acid (made by quickly adding 10 ml. of concentrated hydrochloric acid to 19 mg. of silver as silver nitrate, dissolved in 1 ml. of water). The solution is thoroughly mixed and diluted to a volume of 2 liters with water. The solution is then seeded with 1 mg. of silver as silver nitrate in a few drops of water. The mixture is allowed to digest overnight at room temperature, and then filtered. The silver chloride precipitate is washed with 1% nitric acid to avoid peptization, and the walls of vessel *A* are washed. The silver chloride is dissolved in about 3 ml. of concentrated ammonium hydroxide, then reprecipitated as the chloride by addition of a drop or two of hydrochloric acid. The solution is then made acidic with 6*M* nitric acid. The silver chloride is again filtered and washed with 1% nitric acid and dissolved in 3 ml. of concentrated ammonium hydroxide for the final product.

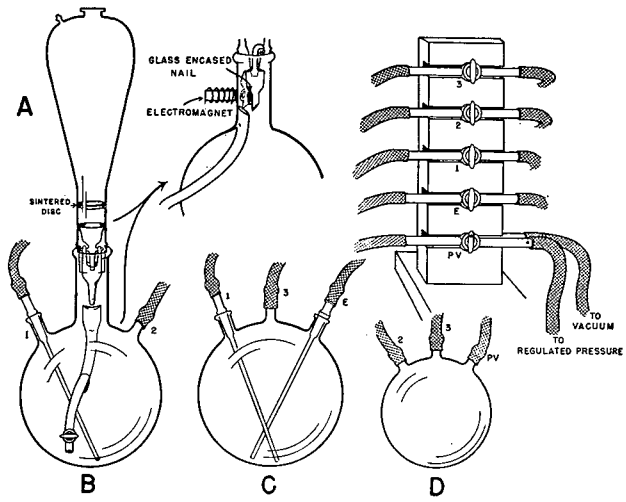

Figure 1. Apparatus for separation of silver-111 from palladium

If silver nitrate is desired as the final product, the silver chloride precipitate, after washing, is dissolved in a minimum amount (less than 1 ml.) of concentrated ammonium hydroxide. One milliliter of 1*N* sodium hydroxide is then added and the solution is heated for about 10 minutes to expel ammonia and to complete the metathesis to silver oxide. The oxide is collected on the filter, washed with water, and then dissolved in the desired amount of nitric acid. The product solution is withdrawn through the product tube.

ACKNOWLEDGMENT

This work was carried out in part under a project sponsored by the U. S. Atomic Energy Commission. Appreciation is expressed to R. G. Wymer, E. L. Alexander, and F. L. Boyer for help in preliminary studies and to Thomas Marshall for help in design and fabrication of glassware.

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Direct Volumetric Determination of Total Zinc in Mixed Paint Pigments with Ethylenediaminetetraacetic Acid—Correction

The authors regret an error in the paper "Direct Volumetric Determination of Total Zinc in Mixed Paint Pigments with Ethylenediaminetetraacetic Acid" [ANAL. CHEM. **27**, 2005 (1955)]. It is erroneously stated that the indicator Eriochrome Black T is a magnesium complex of a hydroxylated azo dye. Eriochrome Black T is the sodium salt of 1-(1-hydroxy-2-naphthylazo)-5-nitro-2-naphthol-4-sulfonic acid.

M. H. SWANN

Calibrating Commercial Test Equipment Scales for Results from Linear Reactions

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A device is presented for transforming linear reactions of the type $y = mx + b$ to the types $y = x$, $y = m'x$, or $y = m'x + b'$, where m' and b' are constants conveniently chosen to save time in computation. The transformation is achieved by varying the volume ratio of reacting materials in accordance with a generalized formula. This device is especially recommended for setting up instruments for routine test determinations involving a large number of samples.

WHEN routine analytical procedures are established for a large number of samples, care is usually taken to diminish the amount of laboratory work. Volumes of aliquots are chosen which can be conveniently and readily measured by available equipment, such as volumetric flasks and transfer pipets. Computation of results is then adapted to the method. However, this usually results in repeated graph readings or prolonged calculations, which take almost as much time as the analytical procedure itself. The object of this paper is to show how, by using fractions instead of whole volumetric units, final calculations of results may be simplified or even eliminated.

Analyses of thousands of leaf samples for copper residue were carried out during extensive studies on the coverage and weathering of Bordeaux fungicide on banana leaves.

Leaf samples A square inch in area were extracted with B ml. of $0.05N$ hydrochloric acid. A 1-ml. aliquot of the extract and 1 drop of ammonium hydroxide (1 to 1) were added to 20 ml. of sodium diethyldithiocarbamate reagent (0.01% aqueous solution). The resulting absorbance was measured in the Fisher Electrophotometer, using a blue filter (No. 425). This instrument is equipped with a logarithmic scale graduated into 100 units covering the range of absorbance from 0 to 1.0. Because the reaction followed Beer's law, a linear equation of the type $y = mx + b$ (Figure 1,A) was obtained, x being the copper concentration in the aliquot expressed in parts per million, and y the number of absorbance scale units (each scale unit = 0.01 absorbance unit). The blank reading varied slightly from day to day and separate graph plottings were necessary for each determination. Each value ($x°$) was determined directly from the graph or by substituting the corresponding absorbance ($y°$) in the equation $y = mx + b$. This value ($x°$) was further multiplied by the factor B/A in order to express results in micrograms of copper per square inch of leaf surface.

The following modifications of the experimental procedure made these computations unnecessary.

1. By trial and error the volume of aliquot was so adjusted that, when the instrument was "zeroed" with the blank, a 50-p.p.m. standard resulted in an absorbance of exactly 0.50. This was done by making use of an adjustable automatic pipet (Schaar & Co., 754 West Lexington St., Chicago 7, Ill.)
2. The factor B/A was brought to unity by having the number of milliliters of extracting solution equal the number of square inches in the total area of sample, which was kept constant with a standard sampling device.

When the instrument readings were plotted against the final results, the $y = x$ line, shown in Figure 1,B, was obtained. Under these conditions the scale reading on the instrument was numerically equal to the final result and computation was eliminated.

This method is applicable to other fields of instrumental analysis, as long as there is a linear relationship between concentration and the instrument dial reading. The only requisites for obtaining the $y = x$ line are that the blank reading be equal to zero on the instrument dial, and the number of units of the standard be numerically equal to the scale reading. Should it become impossible to zero the blank, it may be adjusted to read some round number such as 10 or 20. After the slope is transformed to unity, this number may be mentally subtracted from the instrument reading to give the required result.

In certain instances transformation of the slope to unity may lead to lower sensitivity. In this case, the slope value may be adjusted to any whole number by which the instrument reading may be easily multiplied to obtain the final result. For example, such equations as $y = 11x + 5$ and $y = 2.25x$ lose considerably in sensitivity when transformed to the $y = x$ line. These equations may be conveniently transformed into $y = 10x$ and $y = 2x$ without serious loss in sensitivity. The final results are then obtained by dividing the instrument scale reading by 10 and 2, respectively.

A similar transformation simplifies computation for reactions which are represented by lines with a negative slope. For example, colorimetric determination of some chemical constituent may depend on the degree of decoloration produced in the reagent, which is directly proportional to the concentration of this constituent. The reaction line is then similar to the one shown in Figure 2,A, represented by the equation $y = b - mx$. In this case, the slope may be adjusted to -1, and the constant b to any round number from which the scale reading is then subtracted to obtain the final result. Figure 2,B, illustrates a convenient form into which the $y = b - mx$ line may be transformed. By adjusting the blank to read 1.0 absorbance (100 scale units), and the standard of 50 p.p.m. to read 0.5 absorbance, the equation becomes $y = 100 - x$. In this case, the answer is obtained by subtracting the number of scale divisions from 100.

A general formula has been derived to eliminate the trial and error method for determining the new volume ratio of reacting materials for the desired transformation in slope. This general formula, based on a colorimetric reaction following Beer's law, is equally applicable to any other linear reaction.

Let V_a be the volume of the aliquot of a solution in which the concentration of compound C is to be determined colorimetrically. Let V_r be the volume of the reagent or reagents to which the aliquot is added, and with which compound C reacts to produce a color proportional in absorbance to the concentration of this compound. Let the sum of the volumes V_a and V_r be denoted by V_s.

If absorbance is plotted on the ordinate and the concentration of compound C on the abscissa, the resulting equation may be expressed as

$$y = mx + b \qquad (1)$$

with m being the slope and b a constant depending on the reading of the blank. Let V_a' be the new volume of the aliquot which, when added to the same volume of reagents V_r, results in a new equation with the slope equal to unity, represented by

$$y = x + b \qquad (2)$$

If the apparatus is initially zeroed with distilled water, let V_r ml.

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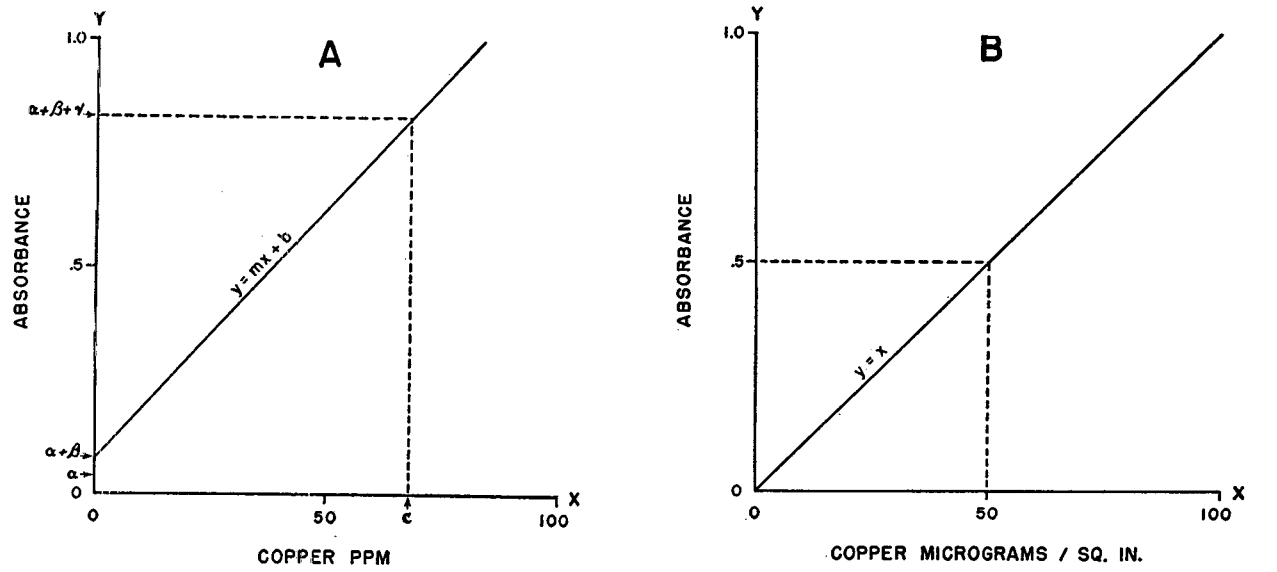

Figure 1. Typical linear reaction

A. **Positive slope obtained in analysis of copper with electrophotometer** *B.* **Modified to eliminate computations of final results**

of the reagent be completed to V_s ml. in the three following ways and with these resulting absorbances:

Absorbance equal to α when completed with distilled water
Absorbance equal to $\alpha + \beta$ when completed with the solution used for the preparation of the blank
Absorbance equal to $\alpha + \beta + \gamma$ when completed with the same solution to which compound C was added at concentration c

The meaning and relationship of α, β, γ, and c are illustrated graphically in Figure 1,*A*.

In this case, the value α is due to the absorbance of the reagent. The value β is due to the color of impurities of the solution with which the blank is prepared or to a certain amount of the compound C initially present in the blank. The value γ represents the net effect on the absorbance from the reaction of the compound C with the reagent.

The general Equation (1) of the initial reaction may now be rewritten by replacing the parameters m and b with α, β, γ, and c, using the two-point form equation of the straight line:

$$\frac{y - y_1}{x - x_1} = \frac{y_2 - y_1}{x_2 - x_1} \tag{3}$$

Substituting $(c, \alpha + \beta + \gamma)$ for (x_2, y_2) and $(o, \alpha + \beta)$ for (x_1, y_1) the equation of the initial reaction becomes

$$y = \frac{\gamma}{c} x + (\alpha + \beta) \tag{4}$$

The initial slope is then given by $m = \frac{\gamma}{c}$, from which c may be expressed in terms of the initial slope and γ; thus:

$$c = \frac{\gamma}{m} \tag{5}$$

If the volume of the aliquot is now reduced to V'_a ml. from the original V_a ml., the effect on the absorbance due to the solution used in the preparation of the blank is reduced in proportion, and is given by

$$\beta \times \frac{V'_a}{V_a}$$

Similarly, the effect on the absorbance due to compound C at the given concentration becomes equal to

$$\gamma \times \frac{V'_a}{V_a}$$

The constant α, which depends upon the absorbance of the reagent, remains the same, because any change produced in the reagent is included in the value γ, which represents the net result due to the addition of compound C. The final absorbance, however, is increased due to the more concentrated solution resulting from the reduced volume V'_a of the aliquot. The correction factor by which the final absorbance is multiplied to obtain the actual reading is consequently equal to

$$\frac{V_a + V_r}{V'_a + V_r}$$

The absorbance corresponding to concentration c for the reduced volume V'_a accordingly becomes

$$\left[\alpha + (\beta + \gamma)\frac{V'_a}{V_a}\right] \times \frac{V_a + V_r}{V'_a + V_r}$$

and the absorbance corresponding to the blank solution, when the new volume V'_a is used, is equal to

$$\left[\alpha + \beta \frac{V'_a}{V_a}\right] \times \frac{V_a + V_r}{V'_a + V_r}$$

Having established these two points of reference of the transformed equation, this equation may now be expressed by substituting the coordinates of these points into the two-point form Equation 3.

After replacing c by $\frac{\gamma}{m}$ according to Equation 5, this transformed equation is finally expressed as follows

$$y = \frac{V'_a}{V_a} \times \frac{V_a + V_r}{V'_a + V_r} \times mx + \left(\alpha + \beta \frac{V'_a}{V_a}\right) \times \frac{V_a + V_r}{V'_a + V_r} \tag{6}$$

Because this equation is equivalent to Equation 2 by hypothesis, its slope must be equal to 1.

Consequently

$$\frac{V'_a}{V_a} \times \frac{V_a + V_r}{V'_a + V_r} \times m = 1$$

By making

$$\frac{V_a}{V_r} = F \quad \text{and} \quad \frac{V'_a}{V_r} = F'$$

after a simple transformation the following formula is obtained

$$F' = \frac{1}{m\left(1 + \frac{1}{F}\right) - 1} \tag{7}$$

This formula permits computation of the new aliquot to reagent volume ratio, F', that results in the linear equation with the

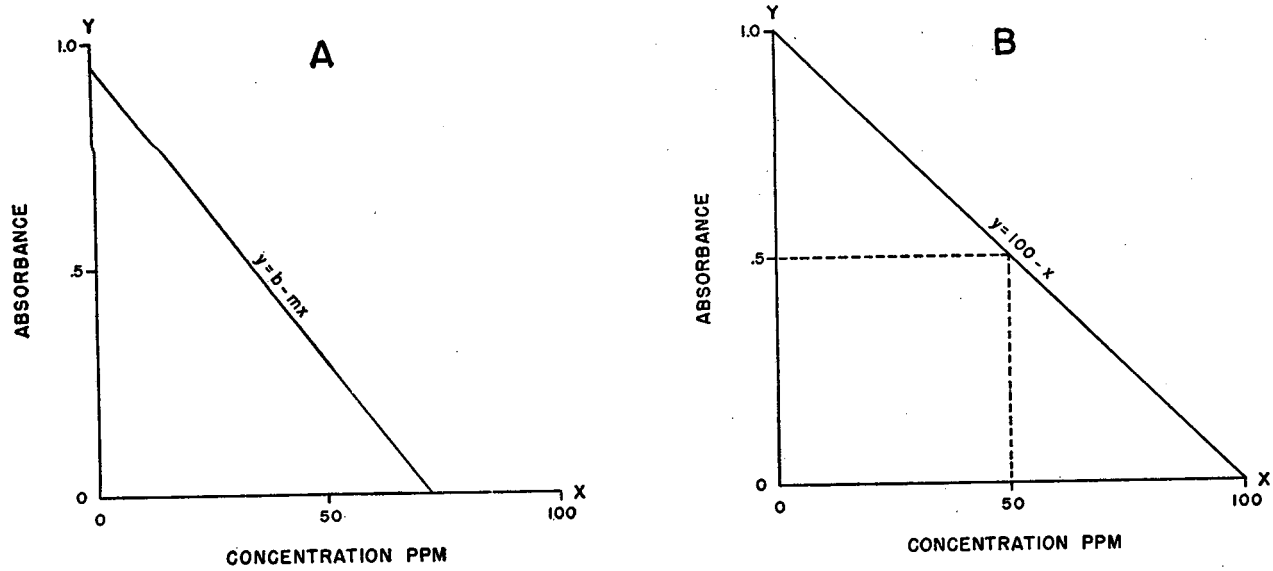

Figure 2. Typical linear reaction

A. **With negative slope** *B.* **Modified to simplify computations**

slope equal to unity, when the initial slope, m, of any linear reaction and the initial aliquot to reagent volume ratio, F, are known.

By similar reasoning, it may be shown that the same formula is applicable to reactions represented by lines with a negative slope.

It is evident from this formula that the slope of a linear reaction is independent of the initial absorbance due to the blank reading. Because the slope of the line is not changed by adjusting the blank reading to zero or any other number, this formula is applicable to any linear reaction, whether or not it passes through the origin.

Relationship 7 also shows that the transformation of any slope to unity is possible, provided the denominator is positive. This condition is always satisfied if the initial slope is greater than 1, irrespective of F value. For reactions with a negative slope this is true when the value m is algebraically less than 1.

When it is desired to change the slope to any number other than 1—e.g., 2, 10, $^1/_{10}$, etc.—the following generalized formula is obtained by equating the coefficient of x, in Equation 6, to m' rather than to unity:

$$F = \frac{m'}{m\left(1 + \frac{1}{F}\right) - m'} \qquad (8)$$

where m' is any slope value desired, the values m, F', and F are as previously defined. The transformation made according to Relationship 8 is easily achieved when the initial slope m is slightly greater than m' for reactions with a positive slope, and when it is slightly less than m' for reactions with a negative slope. This condition is not indispensable, provided that $m\left(1 + \frac{1}{F}\right)$ is numerically greater than m'.

The practical application of Formula 7 may be illustrated by the original example of the copper reaction. When the blank was adjusted to zero, the absorbance corresponding to 50 p.p.m. of copper was 0.55. The initial slope was consequently equal to 55/50. The ratio F was 1/20, since a 1-ml. aliquot was used for every 20 ml. of reagent. In order to bring the slope to unity the new ratio, F', may now be calculated

$$F' = \frac{1}{\frac{55}{50}(1 + 20) - 1} = 0.04525$$

Hence the exact new volume of the aliquot that gives the required transformation in the slope, all other conditions being kept constant, is given by

$$V_a' = V_r F' = 20 \times 0.04525 = 0.905 \text{ ml.}$$

If a considerable change in the F value is required to achieve the desired transformation of the slope in any given reaction, it may become imperative to change the concentration of the reagents to avoid excessive wastes or abnormalities that may result from offsetting the proportion of the reacting materials.

Thus if in some reaction the aliquot to reagent ratio were the reverse of the ratio in the reaction just discussed—i.e., if 20 ml. of aliquot were added to 1 ml. of reagent—the F' value would be entirely different, even if the initial slope had been the same:

$$F' = \frac{1}{\frac{55}{50}\left(1 + \frac{1}{20}\right) - 1} = 6.45$$

This means that the volume of the aliquot must be 6.45 times greater than the volume of the reagent; or inversely, that the volume of the reagent must be 6.45 times less than the volume of the aliquot. For each 20 ml. of aliquot, therefore, 3.1 ml. of reagent must be added to bring the slope to unity. Since this quantity is 3 times more than is necessary for the actual reaction, the reagent may be diluted about three times and then 3.1 ml. of the diluted reagent may be used to achieve the same end.

Once the volume ratio is found by means of the foregoing formulas, the trial and error method may still be used for minor adjustments which are usually necessary because of the variation inherent in any reaction.

If this technique is followed, analytical procedures involving linear reactions may be designed so that direct instrument reading or some easily calculated proportion of it becomes numerically equal to the concentration which is being determined. Only minor adjustments in the volume of reagents or solutions are necessary to utilize the instrument readings conveniently.

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Analysis of Hydrocarbon Fraction of Gilsonite

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It has been shown that 4 to 8% of hydrocarbon material can be separated from gilsonite by chromatography. This material has been named the "giltene fraction" of gilsonite. The giltenes contain 30 to 40% of aromatic molecules and 60 to 70% of naphthene molecules.

A REVIEW of the literature on gilsonite (*1, 3, 5, 14*) revealed a lack of information on what could be accomplished by chromatographic techniques. Such an investigation was therefore undertaken. Chromatographic studies have been made on Canadian tar sands (*9*), LaBrea tar (*23*), a high-boiling shale oil distillate (*6*), and asphalt (*8*).

In this investigation the initial separation of gilsonite was effected using Magnesol as the adsorbent with Celite added to increase permeability of the bed. The low cost and ready availability, as well as its demonstrated efficiency in the separation of carbohydrate derivatives (*15*) and products of pyrolysis of gilsonite (*20*), made the adsorbent attractive. Because gilsonite is highly soluble in benzene, its use in this chromatographic procedure appeared desirable.

REAGENTS AND APPARATUS

The following reagents are used:

Magnesol, a hydrated magnesium acid silicate, Westvaco Chlorine Products Co., South Charleston, W. Va.

Celite, a diatomaceous earth, Johns-Manville Co., New York, N. Y.

Silica gel No. 12, 28 to 200 mesh, Davison Chemical Corp., Baltimore, Md.

2,2,4-Trimethylpentane, spectral grade, Distillation Products Industries, Rochester, N. Y.

The equipment needed is a Beckman spectrophotometer, Model DU; a Todd precise fractionation assembly with fractionating column, 5 mm. in inside diameter, Monel metal, spiral-packed; and a Perkin-Elmer recording infrared spectrophotometer, Model 21.

EXPERIMENTAL

Process Gilsonite. A 600-ml. sintered-glass Büchner funnel was filled three-quarters full (dimensions of adsorbent: 70-mm. depth, 95-mm. diameter) with an intimate mixture of Magnesol-Celite, 5 to 1 by weight. The funnel was repeatedly tapped with a rubber mallet and subjected to aspirator vacuum with more tapping. A large circle of filter paper was placed on top of the adsorbent to prevent erosion.

A solution of 20 grams of Bonanza gilsonite in 90 ml. of benzene was prepared by agitation in a Waring Blendor for 3 minutes. The Magnesol column was prewetted with enough benzene to cover the adsorbent to a depth of at least 1 cm., and the gilsonite solution was then quickly poured into the supernatant benzene. It is essential that the adsorbent be covered by benzene at the moment the gilsonite solution is poured into the funnel and that the aspirator be in operation continuously.

The Blendor was washed six or seven times with 90-ml. portions of benzene, and the washings were used to develop the column. All of the black material of the gilsonite adhered to the adsorbent; the effluent was a clear light brown at first, gradually becoming colorless. Between 500 and 600 ml. of effluent was sufficient to remove all of the light-brown oil from the Magnesol column.

The benzene was removed from the effluent at atmospheric pressure, leaving an oil which was cooled and dissolved in 25 ml. of petroleum ether (boiling point 35° to 60° C.). The resulting solution was placed on a 35 × 100 mm. column of the same adsorbent, which had been prewet with petroleum ether. The column was developed with more of the same solvent until 150 ml. of effluent had been collected. Removal of solvent from this effluent at atmospheric pressure gave 1.2 to 1.6 grams of an oil fraction, designated as "giltenes," and representing 6 to 8% of the gilsonite. The yield of giltenes from Eureka gilsonite was 4 to 5% when the same procedure was followed.

Giltene Components. When 75 grams of giltenes had been collected, they were fractionated at a pressure of 0.06 ± 0.03 mm. of mercury into nine overhead cuts. Most of the cuts were separated further into aromatic and nonaromatic portions by the use of silica gel adsorption (*22*). A 2-gram charge of a giltene fraction was separated effectively on a 35 × 130 mm. column of silica gel by eluting with 250 ml. of low-boiling petroleum ether, which removed the nonaromatic portion completely. The aromatics were then eluted with 250 ml. of ethyl ether or benzene. Solvent removal from all fractions was effected on a steam bath. The samples were then placed in a vacuum desiccator until constant weight was obtained. Material recovery ranged from 91 to 98%. These separation data are found in Table I.

Densities, molecular weights, and refractive indices were determined when enough material was available. The density measurements were made in a microvolumetric flask and required 0.60 ml. of material. The molecular weights were obtained cryoscopically in benzene and required about 0.25 gram of sample. Colored compounds contained in the aromatic fractions made accurate measurements of indices of refraction impossible, although no difficulty was encountered with the nonaromatics. These data are summarized in Table II.

Ultraviolet Spectra. Because ultraviolet spectroscopy can provide valuable information concerning types of aromatic compounds contained in a mixture, ultraviolet curves were obtained on most of the fractions using 2,2,4-trimethylpentane as the solvent and blank. The spectra of the nonaromatic portions between 220 and 340 mμ had no significant absorption peaks. The aromatic portion of the first cut gave a spectrum with peaks at 224, 272, 277.5, and 321 mμ. As the molecular weight of the aromatic samples increased, these peaks shifted slightly to a higher wave length and the two middle peaks tended to coalesce. The ultraviolet spectra of the aromatic portions of cuts 7 to 9 exhibited a small but distinct peak at 254 to 257 mμ in addition to the other peaks described above.

Table I. Fractionation of Giltenes by Distillation and Silica Gel Adsorption

Cut No.	B.P., ° C. at 0.06 ± 0.03 Mm. of Hg	Wt. %	% Aromatics	% Naphthenes	% Recovery
1	54–67	2.0	29.6	66.6	96.2
2	67–77	1.8	...	...	...
3	77–82	1.9	...	...	...
4	82–85	1.5	35.2	58.3	93.5
5	85–88	1.8	35.5	56.1	91.6
6	88–94	2.5	33.0	60.1	93.1
7	94–101	2.0	40.0	58.1	98.1
8	101–110	3.6	43.3	53.2	96.5
9	110–138	7.6	46.2	50.9	97.1
Residuum		74.0	35.0	62.9	97.9
		98.7			

Table II. Physical Constants and Elemental Analyses of Giltene Fractions

	Aromatic Portion						Naphthenic Portion				
Cut No.	Density, 25° C.	Molecular weight	n_D^{25}	% carbon	% hydrogen	% sulfur	Density, 25° C.	Molecular weight	n_D^{25}	% carbon	% hydrogen
1	...	...	...	...	...	...	0.829	198	1.4551	86.08	13.94
4	...	...	1.54	89.18	10.69	...	0.848	237	1.4635	...	...
5	...	...	1.54	...	...	...	0.844	241	1.4623	...	...
6	0.949	228	1.535	88.72	10.32	0.78	0.846	267	1.4637	86.38	13.72
7	0.955	234	1.535	88.79	10.22	1.29	0.855	276	1.4669	86.52	13.76
8	...	267	1.53	89.83	10.07	...	0.871	...	...	...	...
9	0.959	...	...	...	...	...	0.875	...	...	...	...

Table III. Hydrocarbon Comparison

	Pyrolyzed Gilsonite Hydrocarbon	Decane (*19*)	1-Decene (*19*)
Mol. wt.	141	142	140
Density, 25° C.	0.733	0.726	0.737
n_D^{25}	1.4124	1.4097	1.4191
Bromine No. (*2*)	35	0	114

Table IV. Characterization of Nonaromatic Hydrocarbons from Giltenes

Cut No. of Naphthenic Portion	Molecular Weight	d_4^{20} [a]	Weight % Ring (*13*)	Pairs H Atoms Deficient from C_nH_{2n}	n_D^{20} [b]	Refractivity Intercept ($n - d/2$)
1	198	0.833	62.0	0.402	1.4575	1.041
4	237	0.851	62.9	...	1.4653	1.040
5	241	0.847	58.7	...	1.4641	1.041
6	267	0.849	54.1	0.894	1.4655	1.041
7	276	0.858	59.2	0.915	1.4687	1.040

[a] Calculated from densities measured at 25° C. (*10*).
[b] Calculated from n_D^{25}. $\Delta n = 0.6\Delta d$ (*11*).

Infrared Spectra. The presence of any unusual molecular species may be indicated by infrared spectroscopy. However, the spectra of certain fractions provided no significant information. The complexity of the mixtures and the lack of sufficient resolution resulted in curves which contained absorption peaks which are common to most hydrocarbon mixtures.

Molecular Complexes. Many derivatives of naphthalene and more highly condensed aromatic rings form complexes with picric acid (*4*) and 2,4,7-trinitrofluorenone (*17*). All of the aromatic fractions gave a distinct red coloration when mixed directly with crystalline picric acid or when dissolved in a benzene solution of picric acid. All attempts to isolate solid derivatives with either picric acid or 2,4,7-trinitrofluorenone resulted in failure. The nonaromatic portions of the giltenes gave no observable coloration with picric acid.

Hydrogenation Study. In order to test for olefinic unsaturation, various giltene fractions and the total giltenes were subjected to a hydrogenation procedure, using Raney nickel W-5 (*18*) as the catalyst. No hydrogen absorption was observed in any instance. Under the same conditions (room temperature and 1.3 atmospheres of hydrogen), camphene, as well as a mixture of camphene and total giltenes, was reduced smoothly. The catalyst was not poisoned by the small amount of sulfur found in the aromatic portion of the giltenes.

Urea Adduction. This procedure permits the separation of compounds containing seven or more consecutive methylene carbon atoms from branched cyclic compounds (*24*). Thirty grams of residuum from the vacuum distillation of the giltenes, 30 grams of urea, 50 ml. of low-boiling petroleum ether, and 5 ml. of methanol were stirred mechanically for 90 minutes at room temperature (25° C.). The urea-adduct was filtered, washed with 300 ml. of petroleum ether, and dried thoroughly in air. When dissolved in 200 ml. of warm water, the adduct released a small amount of waxy solid, which was dissolved in 100 ml. of low-boiling petroleum ether. The resulting solution was dried over anhydrous calcium chloride, and the solid was recovered by solvent removal on a steam bath. A heavy, faintly yellow wax in an amount of 0.06 gram remained with a melting point of 57° to 63° C.

Oxidation and Nitration. Several of the giltene fractions were subjected to oxidation and nitration procedures in an attempt to effect added separation and to isolate individual compounds. Nonaromatic fractions were recovered unchanged when subjected to the action of aqueous potassium permanganate at the reflux temperature for 4 hours. Oxidation products derived from the aromatics were obtained in insufficient quantities for further work.

Representative aromatic fractions were nitrated using glacial acetic acid and 70% nitric acid, 2 to 1 by volume. The yellow semisolid nitration product could not be crystallized. Chromatography of this material on Magnesol yielded several indistinct zones, none of which contained crystalline material. The contents of the leading zone gave a positive carbonyl test with 2,4-dinitrophenylhydrazine, but the resulting hydrazone was not crystalline even after being chromatographed on Magnesol.

Urea Adduction of Gilsonite Coker Distillate. Gilsonite coker distillate, boiling point 120° to 250° C., from which phenols, pyrroles, and amines had been removed (*20*), was processed with urea and methanol as described under Urea Adduction. A 2.5% yield of colorless oil was obtained, which was fractionally distilled under 2-mm. pressure. The first cut obtained is compared with decane and 1-decene in Table III.

DISCUSSION

Chromatography of gilsonite, using Magnesol as the adsorbent, enabled the separation of a viscous yellow oil in an amount of 6 to 8% by weight from Bonanza gilsonite, and 4 to 5% from Eureka gilsonite. This material has been named the "giltene fraction". It was fractionally distilled into nine cuts, and further separated into aromatic and nonaromatic portions. Physical and chemical determinations were made on these portions. Olefinic unsaturation was demonstrated to be absent in the total giltenes as evidenced by lack of hydrogen absorption.

Using the correlations developed by Lipkin, Martin, and Kurtz (*13*), the various cuts of the nonaromatic portion were calculated to contain between 54.1 and 62.9% by weight of ring carbons (Table IV). The hydrogen and carbon analyses show the ratio of these atoms to be less than 2. The refractivity intercepts, $n - d/2$, for these fractions vary between 1.040 and 1.041. Ward and Kurtz (*21*) determined an average figure for the refractivity intercept of saturated monocyclic naphthenes to be 1.0395. An average figure for paraffins is 1.0455. These data provide strong evidence for the predominance of cyclic compounds in these saturated fractions. Evidence for the absence of aromatic compounds in these naphthenic fractions was provided by the absence of absorption peaks between 220 and 340 mμ.

Cuts 6 and 7 of the aromatic portion were subjected to the correlation of Martin and Sankin (*16*). Specific dispersions were calculated from density, refractive index, and molecular weight by the method of Lipkin and Martin (*12*). Cut 6 was found to contain 1.2 aromatic rings and 1.3 naphthenic rings per molecule, and cut 7, 1.1 and 1.5 rings, respectively. These values are subject to more than the usual experimental error, since refractive indices could not be determined with accuracy because of color contained in the fractions. The refractivity intercepts of these fractions are about 1.06, which corresponds to cyclic aromatic molecules, or molecules containing both condensed aromatic and naphthene structures.

The ultraviolet spectra of the aromatic portions closely resembled those of alkylnaphthalenes not only in over-all appearance, but also in the location of the most prominent peak near 224 mμ (*7*). The additional peak in the vicinity of 255.5 mμ, occurring only in the spectra of the higher fractions, suggested the presence of hydrocarbons related to anthracene or phenanthrene. The deep red coloration resulting from the mixing of the aromatics with picric acid substantiated the occurrence of substituted aromatics in all of the aromatic fractions. Oxidation and nitration procedures, as applied to the aromatic fractions, gave but small amounts of derived products and thus did not provide any useful information regarding types of ring structures present.

The giltene residuum was subjected to urea adduction, which enabled the separation of 0.2% by weight (less than 0.02% by weight of gilsonite) of a waxy solid. However, in large molecules which adduct with urea, it is possible to have some branching or even a cyclic entity in addition to the linear carbon requirement. Thus, no definitive statements concerning the structural make-up of these compounds can be made. Urea adduction was not carried out on the nine overhead giltene fractions because of insufficient material.

Urea adduction of a fraction derived from the pyrolysis of gilsonite provided 2.5% of adducts (1.4% by weight of gilsonite degraded). Some of the properties of the lowest-boiling cut of this fraction were determined (Table III). The higher content of straight-chain hydrocarbons in the pyrolyzate suggested that these compounds were formed by cleavage of more complex molecules. It appears unlikely that the pyrolysis conditions would favor the formation of straight-chain hydrocarbons from branched.

Several varieties of gilsonite are available commercially, but almost all of the work described in this paper was done using Bonanza gilsonite, which had a higher giltene content than Eureka gilsonite.

The nonhydrocarbon portion of gilsonite, not considered in this investigation, contains about 2.5% nitrogen and 0.5% sulfur (*1*). Oxygen content is low (0 to 2%) in most samples. The general properties of gilsonite approach that of a high-melting asphalt.

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Determination of Hydrogen in Titanium Metal by Hot Extraction

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A method and apparatus for the determination of hydrogen in titanium metal are described. Samples of sponge, ingot drillings, sheet, or solid stock are vacuum hot-extracted at 900° to 950° C., and the pressure and temperature of the collected gas, known to be pure hydrogen, are measured in a known volume. The hot extraction of sponge, ingot drillings, or thin sheet is generally complete in 15 minutes. Thick solid samples require a longer time to complete the hot-extraction process.

THE titanium industry has from the beginning been faced with the necessity for developing a rapid, accurate method for the determination of hydrogen in titanium metal. Jaffee and Campbell (*2*) and Lenning, Craighead, and Jaffee (*3*) discussed the effect of hydrogen on mechanical properties of α-titanium, and recommended hydrogen levels as low as 0.005% by weight for good impact resistance properties. This paper briefly describes a satisfactory apparatus and procedure for analyses in this concentration range that have been in use in this laboratory for about 2.5 years.

Observations made on the hydrogen-titanium system by various investigators (*10*) suggested that hot extraction in a high vacuum should be an adequate method for separation of hydrogen from titanium. In particular, the equilibrium studies of Gibb and Kruschwitz (*1*) and McQuillan (*6–8*) showed, first, that at temperatures above the 500° to 700° C. range, rate of diffusion of hydrogen into or out of titanium metal becomes rapid; and second, that at partial pressures below 0.1 micron, only negligible amounts of hydrogen (0.0001% at 950° C., Figure 1) remain at equilibrium in the metal.

Vacuum hot-extraction apparatus have been developed by a number of investigators (*11*), the most complex of which is probably that of Sims and Moore (*9*) for the hot extraction of hydrogen from steel. In this apparatus, a resistance furnace heats the samples to 1050° C. in a vacuum maintained by an automatic Toepler pump. The collected off-gases are transferred to an Orsat tube by a diffusion pump and a two-stage Toepler pump. Forty hours are required to degas a 50-gram sample. All controls are automatic. Another hot-extraction apparatus, described by McGeary (*4*), uses an induction furnace to heat 0.5-gram zirconium samples to 1200° C. A mercury diffusion pump removes the gas, primarily hydrogen, which is evolved from the sample from the furnace section to the collection volume in about 10 minutes. The pressure of the collected gas is measured on a McLeod gage.

The apparatus herein described is similar to that of McGeary, differing principally in the type of furnace used and in the manner of measuring the pressure of the collected gas. As a means of extracting hydrogen from titanium, it offers simplicity, rapidity, and an acceptable degree of completeness.

APPARATUS

Figure 2 shows the apparatus schematically, with individual parts indicated by letters.

Maximum rapidity and completeness of hydrogen removal from titanium both call for the highest temperature practicable short of the metal's melting point during the degassing operation. However, to avoid on one hand the complications and expense of the induction heating equipment, and on the other, the problem of outgassing furnace assemblies of carbon or refractory materials, the furnace tube was made of fused quartz and heated by a Globar furnace. In this assembly, with a furnace temperature of 1050° C., the sample temperature during hot extraction averages about 920° C., and the extraction time is about 15 minutes.

The mercury diffusion pump serves as one boundary of the gas-collecting volume—that is, it is capable of operating efficiently against a fore-pressure greater than the total pressure of the hydrogen accumulated in the collection system. The pump exhausts the furnace tube to 0.05 micron (measured by McLeod gage, not shown in the diagram) by the end of the extraction period.

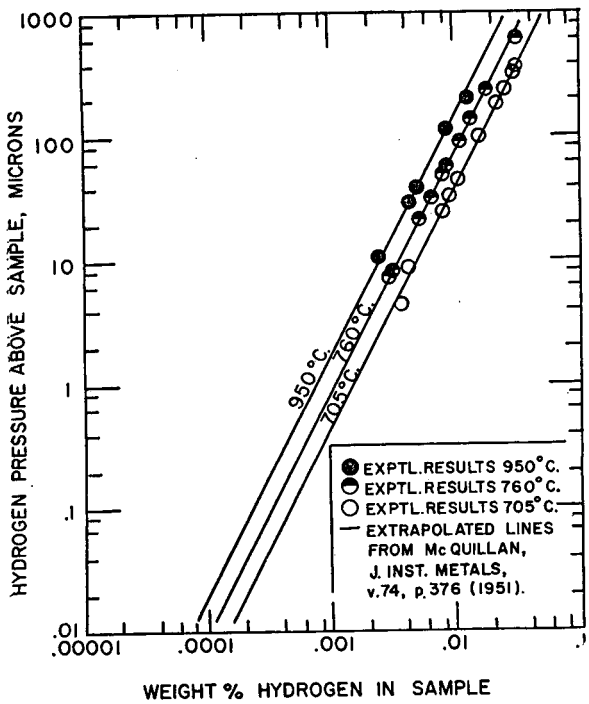

Figure 1. Titanium-hydrogen equilibrium diagram

A small Alnico magnet is used to operate the iron pusher (a small nut and bolt is satisfactory) in the loading arm. A larger magnet is kept in reserve for moving jammed samples or the like. The optical pyrometer has a range of 780° to 1400° C.; direct reading of the sample temperature reveals when the furnace tube is filled to the limit of its hot zone.

The Pirani gage is not necessary for operation of the apparatus but proves extremely useful as a means of following the pump-down of the two parts of the system. A 5-liter bulb at *H* increases the volume of the gas-collection system for samples high in hydrogen. The smaller gas-collection system (1600 ml. in the apparatus described) is otherwise bounded by the diffusion pump, stopcocks *N* and *O*, and the oil manometer. The use of a three-way stopcock at *L* permits the selective exhaustion of either the furnace tube part or the gas-collection part of the system.

PROCEDURE

Solid samples are filed shiny, then freed of grease by washing with C.P. distilled benzene. Powder, drillings, or loose sponge samples are encased in a tin-foil capsule (Baker's purified tin foil is satisfactory). A 3-gram sample for many types of material represents a reasonable compromise between precision and rapidity of the hot-extraction operation. The tin blank is equivalent to about 0.0005% hydrogen on a 3-gram sample.

Impure Kroll sponge samples containing appreciable amounts of magnesium chloride must be vacuum dried at a temperature sufficiently high to decompose the monohydrate (at least 300° C. but less than 400° C., in a good vacuum oven) before hot extraction.

Samples sufficient to fill the furnace tube are placed in the loading arm. A clean furnace tube is joined to the system with Apiezon T vacuum grease, using a minimum of grease to avoid sample contamination. All stopcocks except *P* are opened. Stopcock *L* is set to exhaust the gas-collection system. The mechanical and diffusion pumps are started, and the Globar furnace is turned on. In about 20 minutes the diffusion pumps are operating and the furnace tube is at temperature; then stopcocks *N* and *O* are closed and the system is checked for leaks by observing the rate of pressure increase in the smaller gas-collection volume. The rate should be negligible, representing less than 0.0003% by weight of hydrogen for the sample being analyzed.

After the preliminary check for leaks, the first sample is pushed by magnetic manipulation of the iron bolt from the loading arm into the hot, vertically inclined furnace tube. Hydrogen evolves rapidly and is collected in the small collecting volume. If the pressure threatens to exceed the fore-pressure capacity of the diffusion pump (about 5 mm. of mercury), stopcock *O* is opened to increase the gas-collection volume. The extraction operation is generally complete in 15 minutes except in the case of solid samples, when a 3-gram sample may require 30 to 40 minutes. The final pressure is read after extraction is complete. Knowledge of the hydrogen pressure (corrected for tin-foil blank if tin foil is used), the temperature, the gas-collection volume, and the sample weight permits calculation of the per cent hydrogen. After reading the final hydrogen pressure, stopcock *N* is opened and the accumulated hydrogen pumped out. After about 3 minutes, stopcock *N* is closed and the next sample dropped.

To change a full furnace tube for a fresh one, the furnace is turned off. In about 15 minutes, the samples have cooled to 800° C. Stopcock *M* is closed and stopcock *P* opened slowly. After the tubes have been changed and new samples charged, stopcock *P* is closed, stopcock *L* is turned to the furnace tube section, and pump-down to low pressure follows. Stopcock *L* is then turned back to the gas-collection system, stopcock *M* is opened, and the assembly is again ready for leak check.

DISCUSSION AND RESULTS

Data bearing on the precision and accuracy of the hot-extraction determination for hydrogen were accumulated by miscel-

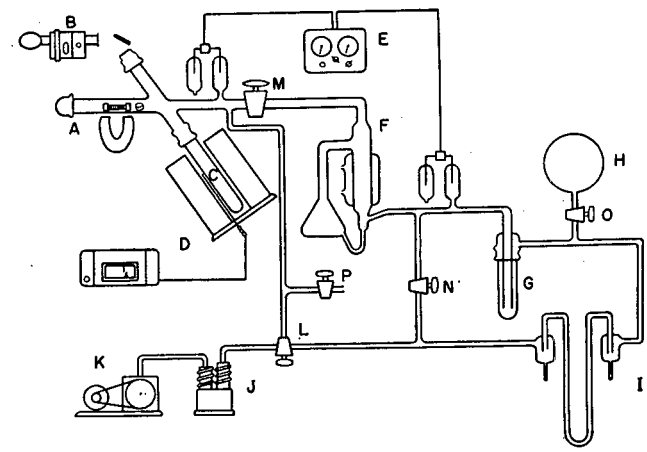

Figure 2. Hot-extraction apparatus

A. Loading arm, 50 cm. × 3.5 cm., with standard-taper joint loading port
B. Optical pyrometer, reflecting mirror, and view port equipped with optical flat for reading sample temperature directly
C. Fused quartz furnace tube, 30 mm. in outside diameter and 60 cm. long, equipped with 40/50 standard taper male joint, which must be concentric with axis of tube
D. Globar tube furnace and temperature controller
E. Two-stage Pirani gage
F. Three-stage mercury-in-glass diffusion pump, Consolidated Vacuum Corp., Type GHG-15
G. Acetone–dry ice cold trap
H. Auxiliary gas-collection volume
I. Oil manometer, filled with dibutyl phthalate
J. Oil-in-meta diffusion pump, Consolidated Vacuum Corp., VMF-20W
K. Megavac or Welch two-stage mechanical pump
L. 10-mm.-bore three-way vacuum grade stopcock
M. 20-mm.-bore vacuum grade stopcock
N, O. 10-mm.-bore vacuum grade stopcocks
P. 4-mm.-bore vacuum grade stopcock

laneous tests upon a variety of titanium metal samples, sponge, sheet, ingot drillings, and solid pieces. The samples in all cases were of unalloyed Kroll process metal. The authors neither sought nor observed any significant variation among these samples in the hot-extraction process, except in length of time for complete extraction, which in thicker pieces of titanium is dependent upon the diffusion rate of hydrogen in titanium.

Rapidity of hydrogen extraction is a function of sample temperature, size, and structure; diffusion pump speed; and length and complexity of path travel by the gas from furnace tube to the diffusion pump. Experience with preliminary models of the apparatus described in this article showed the effect of the gas path to be so considerable as to warrant locating the diffusion pump under the loading arm to minimize the path length. This arrangement is shown in Figure 3. With the present apparatus about 20 determinations can be made per 8-hour shift.

In general, repeatability of the hydrogen determination by hot extraction is probably limited by degree of segregation of hydrogen in the sample rather than by precision of the analytical method. Demonstration of the fact requires preparation of a sample of known uniformity of hydrogen distribution, which has not been attempted by this laboratory. Data obtained from 18 hydrogen determinations on random 3-gram portions of an ingot drillings sample are as follows:

$$\begin{aligned}&\text{Average} = 0.0120\% \text{ hydrogen}\\&\text{No. determinations} = 17^a\\&\text{Standard deviation} = 0.0010\%\\&\frac{\text{Standard deviation}}{\text{Average}} \times 100 = 8.3\%\end{aligned}$$

One result rejected by Chauvenet's criterion.

The composition of gas collected by hot extraction from sponge titanium was determined by oxidation and differential freezing. The average of five determinations was 100.6 ± 0.8% hydrogen.

A rough extension into lower pressure ranges of the equilibrium data obtained by McQuillan was made. Hydrogen was removed from a sample of titanium sheet in small increments with observation of corresponding equilibrium furnace tube pressures (measured by a McLeod gage not shown in Figure 2). The data were plotted, with the assumption made in calculating hydrogen concentrations that negligible hydrogen remained in the sheet at the completion of the extraction operation. The results corresponded closely to an extrapolation of McQuillan's curve, shown in Figure 1. The straight line of the slope, predicted for the case of a true solution of hydrogen atoms in the metal lattice, when extrapolated to the low pressures obtained at the end of a hot-extraction operation at 950° C., indicates less than 0.0003% hydrogen remaining in the sample. Close approach to the equilibrium concentration of hydrogen remaining at the final pressures is indicated by the fact that hydrogen may be repeatedly removed from and returned to sponge, ingot drillings, sheet, or solid samples (by a bypass line not shown in Figure 2) with no change in the total amount recovered on each extraction.

Duplicate samples of sponge and sheet were hot-extracted at 920° C. and 1400° to 1630° C. (in a vacuum fusion apparatus). The results, shown in Table I, show no significant variation in hydrogen content detectable by analysis at these widely separated temperatures.

While the analysis of alloys for hydrogen by this method has not been studied in detail, the method may be expected to apply without modification. McKinley (*5*), in applying the equilibrium pressure method for determining hydrogen to two titanium alloys, found the alloying constituents decreased the solubility of hydrogen in the metal without significantly effecting its diffusion rate through the metal.

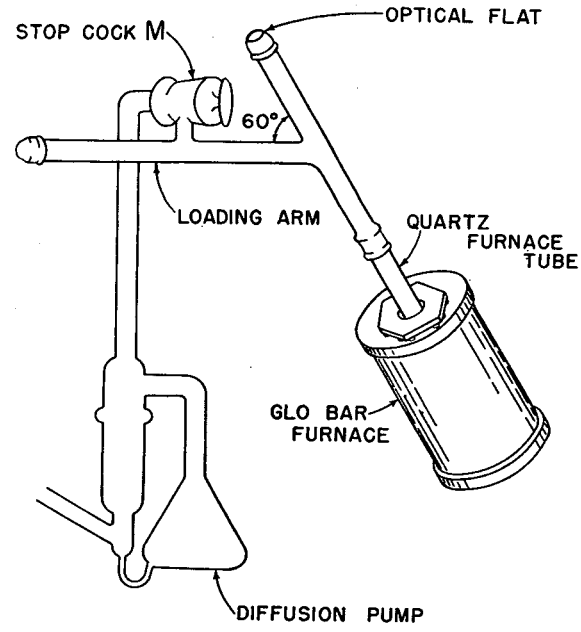

Figure 3. Furnace assembly arrangement

Table I. Comparison of Hot Extraction at 930° C. to Hot Extraction at Temperatures from 1400° to 1630° C.

Trial	Temp., ° C.	% H_2	Trial	Temp., ° C.	% H_2
		Sheet Samples			
1	1400	0.0245	10	930	0.0250
2	1400	0.0245	11	930	0.0248
3	1400	0.0246	12	930	0.0250
4	1500	0.0246	13	930	0.0246
5	1630	0.0242	14	930	0.0248
6	1630	0.0244	15	930	0.0239
7	1630	0.0245	16	930	0.0248
8	1630	0.0246	17	930	0.0246
9	1630	0.0246	18	930	0.0248
	Av.	0.0245			0.0247
	Av. dev.	± 0.00009			± 0.00022
		Sponge samples			
1	1600	0.023	6	950	0.020
2	1600	0.023	7	950	0.023
3	1600	0.020	8	950	0.022
4	1600	0.020	9	950	0.020
5	1600	0.023	10	950	0.021
			11	950	0.022
			12	950	0.023
			13	950	0.021
	Av.	0.022			0.022
	Av. dev.	± 0.0014			± 0.0007

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The authors wish to acknowledge the extensive contributions made by Basil Smith and Katherine Lemmon, who operated the present apparatus and its several predecessors.

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Mixed Perchloric-Phosphoric Acids as Solvents for Iron Ores

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Perchloric and phosphoric acids and mixtures of these acids were studied as solvents for iron ores. The mixture consisting of equal volumes of the two acids was found to be the most satisfactory, and superior to hydrochloric acid. Nearly all iron ores were dissolved in 10 minutes. The iron in the resultant solution, containing an excess of the two acids, was reduced using the Jones (amalgamated zinc) reductor; the reduced iron was titrated directly with permanganate or cerium(IV) sulfate.

THE hydrochloric acid method for the dissolution of iron ores suffers from several pronounced disadvantages. The hydrochloric acid must be kept just below the boiling point to prevent mechanical loss or deposition of iron(III) oxide or chloride on the walls of the vessel. This deposit is sometimes difficult to redissolve even with hot dilute hydrochloric acid.

If the Jones reductor is to be used, it is necessary to remove hydrochloric acid by evaporation with sulfuric acid, because hydrochloric acid causes excessive evolution of hydrogen and results in frequent blocks in the reductor. Evaporation with sulfuric acid often results in the formation of anhydrous iron(III) sulfate that is difficult to redissolve.

When an appreciable amount of silica is present in the ores, hydrochloric acid usually does not remove all of the iron. A sodium carbonate fusion or a hydrofluoric acid treatment is then necessary to obtain all of the iron in soluble form.

Each of the above factors tends to give low results and to prolong the time for completing an analysis.

The method for analysis presented here is believed to be superior to the hydrochloric acid method in that it is less time-consuming and less subject to errors.

The ore is rapidly dissolved by gently boiling with a mixture consisting of equal volumes of 72% perchloric acid and 85% phosphoric acid. After being diluted with distilled water and boiled briefly to distill off the chlorine, the cooled solution is passed through the reductor and titrated with permanganate or cerium(IV) sulfate solution. When the method is well organized, it is possible to perform a complete analysis in less than 30 minutes.

REAGENTS

ORE SOLVENT. Equal volumes of 72% perchloric acid (G. Frederick Smith Chemical Co.) and 85% phosphoric acid were mixed.

STANDARD CERIUM(IV) SULFATE SOLUTION. An approximately 0.1*N* solution was prepared using 58 grams per liter of cerium(IV) hydrogen sulfate (G. Frederick Smith Chemical Co.), then standardized against National Bureau of Standards arsenious oxide. Ceric hydroxide may also be used to prepare the solution.

STANDARD PERMANGANATE SOLUTION. An approximately 0.1*N* solution was prepared and standardized against primary standard sodium oxalate (Mallinckrodt) or National Bureau of Standards arsenious oxide. The method described herein has also indicated that electrolytic iron (Hach Chemical Co.) can be used for standardization.

FERROIN INDICATOR. A 0.025*M* solution was used (G. Frederick Smith Chemical Co.).

PROCEDURE

Weigh into a 500-ml. dry Erlenmeyer flask about 0.3 to 0.35 gram of the ore. Add about 20 ml. of the premixed ore solvent and swirl the flask to disperse the ore. Place a refluxing still head (*1*) in the neck of the flask and heat on a burner or hot plate adjusted so that the acids boil gently. In about 10 minutes, or after the iron is dissolved, cool the flask and contents enough so that about 70 ml. of water can be added without causing the water to boil excessively. Boil for about 2 minutes to remove dissolved chlorine; cool and add about 30 ml. of 1 to 1 sulfuric acid. Pass the cool solution through the Jones reductor at a rate of about 50 to 60 ml. per minute, using suction on the receiving flask if necessary. Add about 0.2 gram of sodium carbonate to the receiving flask before starting the reduction process. This reacts with some of the acid to liberate carbon dioxide, which prevents air oxidation of the iron. Wash the last of the iron solution through the reductor column, using three 25-ml. portions of 1 to 20 dilute sulfuric acid solution and finally three 25-ml. portions of distilled water. Titrate the iron(II) in the receiving flask using either standard permanganate or cerium(IV) sulfate solution. Ferroin indicator is used with cerium(IV) sulfate; rapid stirring is necessary in this titration to prevent the formation of insoluble cerium(IV) phosphate.

EXPERIMENTAL

Dissolution Studies. Boiling perchloric acid (72%) alone failed to dissolve the ores rapidly. Boiling phosphoric acid (85%) gave rapid dissolution, but the silica was converted to a gelatinous form which plugged the Jones reductor so that it became inoperative. An additional disadvantage of phosphoric acid alone was that an insoluble iron(III) phosphate was occasionally formed.

A mixture of 3 volumes of 72% perchloric acid and 1 volume of 85% phosphoric acid was found to give more rapid dissolution than perchloric acid alone, but was not so rapid as phosphoric acid alone. The use of 1 volume of perchloric acid with 3 volumes of phosphoric acid gave very rapid dissolution but was almost as unsatisfactory as phosphoric acid alone.

Equal volumes of perchloric and phosphoric acids resulted in rapid dissolution of the ores but did not give the objectionable form of silica and insoluble iron(III) phosphate in any case.

Table I. Results of Iron Analyses

No.	Sample	No. of Analyses Made	% Fe: Found	% Fe: Av. dev. from av.	% Fe: Orig. anal.	% Fe: Found, dev. from orig. anal.	% Fe: Found, HCl method, corrected SiO_2	Dev. % Fe Found from Silica-Corrected Values
1	Electrolytic Fe[a]	8	100.0	0.06	100.0	0.0	...	...
2	Electrolytic Fe[b]	4	100.1	0.08	100.0	+0.1	...	...
3	NBS 27[a]	4	69.24	0.07	69.26[c]	−0.12	...	...
4	NBS 27[b]	4	69.12	0.06	69.26[c]	−0.14	...	...
5	S. S. Co., 1[a]	4	69.52	0.04	69.52	0.00	...	...
6	S. S. Co., 18[a]	4	51.85	0.01	51.94	−0.09	...	...
7	S. S. Co., 19[a]	8	51.00	0.06	50.95	+0.05	...	...
8	S. S. Co., 2[a]	5	69.23	0.03	68.87	+0.36	69.22	+0.01
9	S. S. Co., 12[a]	4	55.83	0.03	55.61	+0.22	55.80	+0.03
10	S. S. Co., 9[a]	4	57.53	0.02	57.37	+0.16	57.49	+0.04
11	S. S. Co., 5A[a]	4	60.25	0.01	59.96	+0.29	60.22	+0.03

[a] Permanganate as titrant; standardized against primary standard sodium oxalate.
[b] Cerium(IV) sulfate as titrant; standardized against NBS arsenious oxide.
[c] NBS analysis. Average of four steel companies' results is 69.12%.

In all the dissolution studies 20 ml. of the solvent and about 0.3 to 0.4 gram of the ore were used in a 500-ml. Erlenmeyer flask.

Analytical Results. ELECTROLYTIC IRON. About 0.22-gram samples of primary standard electrolytic iron were dissolved, and the iron content was determined using the procedure given above. The result of eight consecutive analyses using permanganate as titrant is given as No. 1 in Table I. No. 2 is the summary result of four consecutive analyses using cerium(IV) sulfate solution as the titrant. The results indicate that the over-all procedure for iron is good and that electrolytic iron is a good and convenient primary standard for the solutions.

NBS No. 27 ORE ANALYSIS. National Bureau of Standards sample 27 was analyzed using the procedure given above. The results of these analyses are given as Nos. 3 and 4 in the table. The values obtained by this method check very closely with t ose of four steel companies for the same sample.

ANALYSES OF STANDARD SAMPLE CO. ORE SAMPLES (Hach Chemical Co., Ames, Iowa). Seven samples for student analysis used in this laboratory were analyzed using the new procedure, and the results are given in column 4 of the table. The values for these samples given in column 6 were determined by the supplier in the following manner: dissolution of the ore sample in hydrochloric acid, removal of hydrochloric acid by fuming with sulfuric acid, dilution, reduction with Jones reductor, and titration with permanganate.

The new method gave good agreement with samples 1, 18, and 19, but only fair agreement with samples 2, 12, 9, and 5A. The authors analyzed sample 12 using the regular hydrochloric acid procedure, and obtained an average *for four analyses of 55.66%*. This is only 0.05% (1 part in 1000) higher than the results given by the supplier.

The supplier's results appeared to be low compared to those obtained by this method only with samples containing moderate to large amounts of silica (Standard Sample Co., 2, 12, 9, and 5A). These samples were then analyzed with hydrochloric acid as the ore solvent, but the silica was filtered off and fused with sodium carbonate. The residue was dissolved in hydrochloric acid and added to the filtrate. The hydrochloric acid was then fumed off with sulfuric acid and the iron was reduced in the regular manner with the Jones reductor. The results of these analyses are given in column 8 of the table. By comparing the results in columns 4, 6, and 8 it is evident that the perchloric-phosphoric acid solvent method gives excellent iron removal from the silica and that the conventional hydrochloric method (column 6) gives low results when considerable silica is present. The new procedure has been used in the sophomore quantitative analysis laboratory at Iowa State College for the past year with good results.

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Determination of 6-Ethoxy-1,2-dihydro-2,2,4-trimethylquinoline in Biological Materials

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A fluorometric method has been devised for measurement of the antioxidant 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline in biological materials in the presence of fluorescing impurities also extracted from tissues by the same method. It is capable of detecting about 0.01 p.p.m. of the antioxidant in a solvent solution. The procedure has proved successful in obtaining approximate analyses of treated alfalfa meal as well as tissues of rats, chicks, and calves employed in chronic toxicity studies. With minor modification, it has been applied in analyses of milk, butter, and eggs from animals fed antioxidant-treated meal.

THE effectiveness of 6-ethoxy-1,2-dihydro-2,2,4-trimethylquinoline as an antioxidant for carotene in dehydrated alfalfa meal was reported in 1950 (*2*). This antioxidant is now commercially available and over 30,000 tons of meal were treated with it in 1954. Feeding tests have shown that its toxicity is low and alfalfa meal treated with the antioxidant for use in poultry feeds is now shipped in interstate commerce. This study required a method of assay for the antioxidant, sufficiently sensitive to permit its detection in animal tissues, eggs, milk, butter, and treated alfalfa meal.

REAGENTS AND APPARATUS

The reagents used were reagent grade acetone, diethyl ether, potassium permanganate, and sodium sulfate. The acetone required redistillation prior to use. Pure-grade iso-octane (99% 2,2,4-trimethylpentane, Phillips Petroleum Co., Bartlesville, Okla.) was found satisfactory. The instrument used for the fluorescence measurements was a Model 12A Coleman photofluorometer with a PC-1 filter (480 mμ) and a BS-1 filter (435 mμ). However, any sensitive photofluorometer used for vitamin assay would be satisfactory if used with comparable filters. Quinine sulfate was used as a fluorometric standard. It was found that 0.06 γ per ml. of quinine sulfate in 0.1*N* hydrochloric acid had a fluorescence equivalent to 0.1 γ per ml. of the antioxidant. A microcup electrical blender was used to macerate the tissue samples.

ANALYSES

Tissue. A sample of about 2 grams is blended in a microblender cup with 50 ml. of freshly distilled acetone. The homogenate is filtered and an aliquot of 10 to 20 ml. of the filtrate is added to 100 ml. of iso-octane. After the acetone has been removed by washing the mixture with water, the iso-octane extract is dried over sodium sulfate. Following appropriate dilution, the total fluorescence of the iso-octane solution is determined. Fifteen milliliters of the extract in iso-octane solution is determined. Then 15 ml. of the extract in iso-octane is shaken with 10 ml. of 0.04% potassium permanganate solution for 1 minute, the potassium permanganate layer is decanted off, and the iso-octane layer is dried over sodium sulfate. The fluorescence of the potassium permanganate–treated iso-octane solution is read and subtracted from the original reading; the difference is the fluorescence attributable to the antioxidant.

Milk. To 25 ml. of thoroughly homogenized milk, 25 ml. of a solution containing 1 part of acetone and 3 parts of iso-octane is added. The mixture is shaken vigorously for 1 minute in a glass-stoppered bottle and transferred to a 500-ml. separatory funnel. The lower layer is discarded and the iso-octane layer is washed with 400-ml. portions of distilled water until all traces of

milk solids have disappeared. The iso-octane layer is dried over anhydrous sodium sulfate to remove turbidity and, after proper dilution, the fluorescence is determined. After treatment with potassium permanganate as described above, the fluorescence is again determined. The difference between the two readings is attributable to the antioxidant.

Table I. Antioxidant Content of Individual Eggs Obtained from Birds Fed Treated Meal

Pen	Antioxidant in Diet, P.P.M.	Antioxidant Found in Egg Yolk, P.P.M. 0.04% $KMnO_4$	0.1N H_2SO_4	1.0N H_2SO_4
1	0	0	0	0
		0	0	0
		0	0	0
		0	0	0
2	7.5	0	0	0
		0.6	0	0
		0	0	0
		0	0[a]	0
3	75	0.8	0.9	0
		0	0	0
		0	0.2	0[a]
		0.9	0	0
		0.9	0	0
		0	0[a]	0[a]
4	750	1.9	0	0
		1.8	0.9	1.1
		2.0	1.3	0.8
		3.1	2.5	2.6
		1.8	0	0
		3.0	1.0	1.0

[a] Deflection readings on fluorometer were higher after treatment with reagent.

Egg Yolk. The egg is broken carefully to avoid rupturing the yolk sac, and the yolk is washed free of egg white with distilled water. (No detectable amounts of antioxidant were observed in egg whites.) Two grams of the yolk is added to 10 grams of anhydrous sodium sulfate in a mortar and the mixture is ground until uniform in appearance. The preparation is allowed to stand for 1 hour in a desiccator, and then a 4-gram sample is blended with 50 ml. of iso-octane for 1 minute in a microblender. The suspension is centrifuged to clarify and the fluorescence is again determined before and after potassium permanganate treatment as described above.

Butter. Ten grams of butter is dissolved in 250 ml. of iso-octane. The solution is shaken with about 20 grams of sodium sulfate and centrifuged to clarify. The fluorescence in iso-octane is then determined before and after permanganate treatment.

Alfalfa Meal. A 1-gram sample of finely ground meal is shaken for 5 minutes with 30 ml. of diethyl ether and filtered, and a 25-ml. aliquot is shaken in a separatory funnel with two successive portions of 0.5N sulfuric acid. The acid extract is made strongly basic with solid sodium hydroxide and is re-extracted with two successive 25-ml. portions of diethyl ether. The ether solution is dried over anhydrous sodium sulfate and the fluorescence is determined directly.

DISCUSSION

The method is based on the measurement of the fluorescence of the compound in petroleum ether or diethyl ether. Preliminary studies showed that the compound fluoresces in visible light and exhibits a very strong blue fluorescence when exposed to ultraviolet light. As little as 0.01 p.p.m. is detectable in a petroleum solvent solution and almost twice this fluorescence is observed in diethyl ether. Plotting the concentration of antioxidant against instrument reading in the range from 0.01 to 0.10 γ per ml. revealed a linear relationship.

Because acetone partially quenches the fluorescence of the antioxidant, it is necessary to transfer to another solvent prior to measurement. Most commercial petroleum ethers contain a small amount of impurity which contributes to the fluorescence under the conditions of the assay. Therefore, it is preferable to use iso-octane, which can be obtained in a relatively pure form, free of fluorescence.

In animal tissue assays, compounds such as riboflavin and niacin, which fluoresce, are also extracted by acetone under the conditions of this assay. For certain tissues and organs, the fluorescence contributed by these compounds is many times greater than that contributed by the antioxidant. Other sources of interference include unknown fluorescing substances formed by reactions of components present in the tissues with the reagents used. Such an effect was also found with certain samples of eggs, particularly eggs which had been stored for a period prior to assay.

For an accurate assay, it is necessary to remove or in some way evaluate the contribution of these impurities to the total fluorescence of the extract. Preliminary attempts to separate them from the extract by chromatography on adsorbents such as alumina or magnesia were unsuccessful, as variable results were obtained. Conditions could not be found which permitted quantitative elution of the antioxidant without removal of some of the other fluorescing materials.

Because the antioxidant is sufficiently basic to permit its quantitative extraction from the iso-octane or ethyl ether extract with dilute sulfuric acid, this avenue appeared to offer promise. Further, it was found that after neutralization with sodium hydroxide, the antioxidant could be transferred back to iso-octane from the aqueous phase relatively free of impurity. Quantitative recovery was obtained by this technique when applied to pure solutions of the antioxidant. However, occasionally small portions of other fluorescing materials were also extracted by the acid from certain tissue and egg samples, resulting in high readings.

Reproducible results were obtained by determining the fluorescence of the extract before and after treatment with sulfuric acid. When the decrease in fluorescence is considered as a measure of the antioxidant extracted by the sulfuric acid treatment, this technique is fairly successful for assays of most tissues and alfalfa meal. However, when an attempt was made to apply the method to eggs from antioxidant-fed birds, occasionally a higher fluorescence value was obtained after sulfuric acid treatment than with the original extract. This observation indicated that certain components in the eggs were reacting with the sulfuric acid to produce additional fluorescing material. Furthermore even 0.1N sulfuric acid (the minimum concentration that would quantitatively extract the antioxidant from iso-octane) occasionally reacted with egg extractives to give enhanced fluorescence (Table I).

As the sulfuric acid treatment was not always reliable, a study was made with potassium permanganate, which has found application in fluorometric assay for riboflavin as a differential fluorescence quencher (*1*). It was found that 0.04% aqueous solution of potassium permanganate shaken with the antioxidant in iso-octane solution completely quenched the fluorescence, whereas the permanganate had no effect on the "nonquinoline" fluorescence in iso-octane extracts prepared from tissues and eggs. When solutions of the antioxidant in iso-octane were homogenized with egg yolk or animal tissue and assayed before and after permanganate treatment, the antioxidant was determined quantitatively. Table I presents data from a typical experiment, in which eggs from antioxidant-fed birds were assayed. Permanganate was compared with 1.0N and 0.1N sulfuric acid for their relative efficiency in extracting the antioxidant. In general, employment of permanganate led to slightly higher results with fewer inconsistencies than did sulfuric acid. The data in this table are representative of those obtained in many of the assays of this study and are typical of the variation found between comparable samples.

The method adopted for the determination of the antioxidant in milk is an adaptation of the method for tissues and eggs. Known concentrations of antioxidant in iso-octane were subjected to the procedure developed for assay of milk and indicated quantitative recovery. Results of a typical experiment are presented in Table II. For this experiment, milk samples were

collected from two cows fed antioxidant equivalent to 10 times the amount expected to be consumed in a cow's daily ration of alfalfa. The data show that the method is sufficiently sensitive to detect the antioxidant in the cow's milk within 2 hours after ingestion.

Table II. Amount of Antioxidant Found in Milk

Time Sample Taken	Cow 380, P.P.M.	Cow 1120, P.P.M.
7 December, A.M.	0	0
8 December, P.M.[a]	0.05	0.02
9 December, A.M.	0.04	0.03
9 December, P.M.[a]	0.19	0.14

[a] Antioxidant given as drench 2 hours before this milking.

Known amounts of antioxidant were added to samples of egg yolk prior to assay and replicate determinations made (Table III). Despite all precautions, occasionally some compounds still interfered with the determination—that is, they were extracted from the tissues along with the antioxidant and were quenched by the permanganate. Thus, chicken liver showed as much as 1.6 p.p.m. in some controls. Accordingly, the values obtained by this method should be regarded as approximate maximum values only. Such errors can be partially resolved by use of a sufficient number of replicated samples. Thus, as in Table IV, the general picture is evident, although obvious inconsistencies are present in the controls.

Preliminary experiments with urine from dogs and rats showed the presence of some component which reacted like the antioxidant when treated with permanganate. Further experiments are necessary to eliminate this interfering material.

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Table III. Recovery of Antioxidant from a Series of Eggs

Antioxidant Added to Egg Yolk, P.P.M.	Total Apparent Antioxidant, P.P.M.	Corrected Antioxidant Value (after $KMnO_4$ Treatment), P.P.M.
3.4	9.7	5.2
	8.4	3.7
	6.9	3.3
	7.5	2.8
	8.4	3.9
0	4.9	0.2
	5.5	0.2
	5.2	0.2

Table IV. Antioxidant Found in Rat Tissues after Feeding for 200 Days

Antioxidant in Diet, %	Rat No.	Tissue Assay, P.P.M. Liver	Fat	Kidney	Muscle
Control	1	0	0	2	0
	2	0	0	0	0
	3	0	0	3	0
0.0125	4	0	0	0	0
	5	0	0	0	0
	6	0	0	0	0
0.20	7	0	11	6	1
	8	0	11	3	0
	9	0	22	3.5	1
	10	0	21	3	1.5
0.40	11	0	74	8	1
	12	0	50	5	1
	13	0	55	4	1

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Automatic *iR* Drop Compensator for Polarographic Use

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An automatic device has been developed which continuously compensates for the *iR* drop in a polarographic cell. The device is particularly useful in the polarographic examination of solutions having very high electrical resistance, and can be used readily in conjunction with any conventional type of polarograph. The compensator completely eliminates time-consuming manual calculations for *iR* drop. It continuously senses the cell current and produces a potential equal to the product of the cell current and the previously measured cell resistance. This compensating potential is then introduced into the cell circuit in such a way as to cancel the effect of the *iR* drop. The compensator has been tested under a variety of conditions with both dropping mercury and solid electrodes, and for both reduction and oxidation processes at the indicating electrode. Reproducibility and accuracy are within the limits of normal polarographic analysis.

MUCH recent development work in organic polarography has been conducted in solutions containing little or no water. In many such cases, the electrical resistances of the cell solutions are very high, resulting in distortion of the current-voltage curves. Interpretation of such curves frequently requires time-consuming, manual correction for the internal resistance of the cell solution. Polarographic analysis, under such conditions, would be greatly facilitated by automatic correction of the entire curve.

In 1952, Ilkovič (*4*) presented a paper in Europe describing an automatic compensator; no details of its construction or operation are available in this country. More recently, Arthur, Lewis, and Lloyd (*1*) described a device for the automatic correction of recorded polarograms, which employed a strip-chart function plotter and two reference electrodes; effective voltage rather than applied voltage was then recorded directly.

An automatic *iR* compensator constructed from standard electrical equipment and suitable for use with any conventional

polarograph is described in the present study. The simplicity of its operation is discussed as well as the accuracy of the results obtained.

PRINCIPLES AND APPARATUS

Interpretation of polarographic current-voltage curves requires that the cell potential be accurately known. If the resistance of the test solution is very small, the potential of the cell is essentially equal to the applied potential. However, if the solution resistance, R, is large, an undesired potential drop occurs in the cell due to the current flow, i, through the high resistance medium. Under these conditions, the real cell potential is determined by subtracting the iR drop from the applied potential. This is usually done by manual, point-by-point correction of recorded or transcribed polarograms.

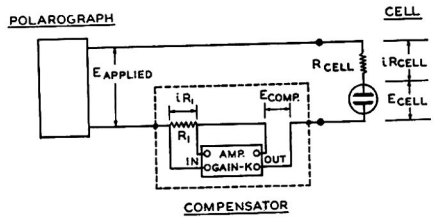

Figure 1. Basic compensating circuit

The device described eliminates manual iR corrections by inserting into the circuit a continuously compensating source of potential, whose potential equals in magnitude but opposes the iR drop in the circuit. Thus, the cell potential is always equal to the applied potential and no corrections of recorded polarograms are required.

A circuit for accomplishing this compensation is shown in Figure 1. A voltage proportional to the undesired iR drop in the cell is developed across a small resistance, R_1, and is amplified in amplifier of gain K to a value which compensates for the iR drop. This amplified voltage is then inserted back into the circuit in opposition to the iR drop.

The relationship between the various parameters required for accurate compensation is readily derived. When the output resistance of amplifier K is made negligibly small, the cell potential can be stated in terms of the other circuit e.m.f. values:

$$E_{cell} = E_{applied} - iR_{cell} - iR_1 + KiR_1 \qquad (1)$$

The requirement for complete compensation is that the cell potential always be equal to the applied potential:

$$E_{cell} = E_{applied} \qquad (2)$$

Subtracting Equation 2 from Equation 1:

$$iR_{cell} + iR_1 - KiR_1 = 0 \qquad (3)$$

and, solving for R_1:

$$R_1 = \frac{R_{cell}}{K - 1} \qquad (4)$$

Complete compensation for any known cell resistance can then be attained readily by fixing either K or R_1 and computing the necessary value of the other from Equation 4. It is convenient to select an arbitrary value of 100 or 1000 for $(K - 1)$. To obtain compensation, it is then merely necessary to measure the cell resistance, divide by 100 or 1000, and adjust R_1 to this value. The resistance, R_1, used in the present study was a calibrated linear potentiometer. The procedure outlined requires that the gain, K, of the amplifier be constant and equal to the arbitrarily chosen value within a tolerance which is better than the desired accuracy of compensation.

Amplifier Characteristics. The performance of the compensation circuit (Figure 1) is largely determined by the characteristics of the amplifier. The requirements to be met by a suitable amplifier are: (1) low "zero" drift, (2) constant gain throughout the frequency pass-band, (3) negligible phase-shift throughout the frequency pass-band, (4) a frequency pass-band of at least zero to 0.1 cycle per second, (5) isolated input and output circuits, and (6) linear operation over an output voltage range equal to the range of iR drop to be compensated. A number of amplifiers, including both electronic and electromechanical types, may be used successfully. A brush direct current amplifier (Model BL-913), a modified General Electric self-balancing potentiometer

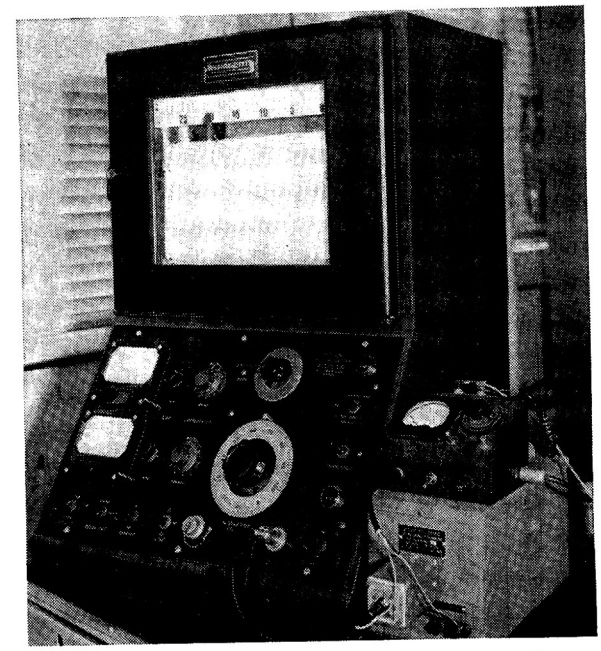

Figure 2. Typical setup of compensator, using commercially available amplifier

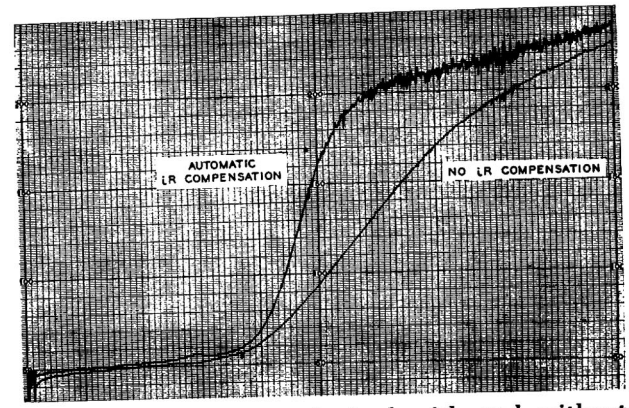

Figure 3. Polarograms obtained with and without automatic iR correction

2.5 ml. of dibutylphenylenediamine in 100 ml. of 1 to 1 iso-octane–isopropyl alcohol, 0.1M in LiCl
Opal wax-impregnated graphite electrode *vs.* Ag-AgCl cathode
1.24 mv. per second; stirred test solution; 19,000 ohms cell resistance

(Catalog No. 8901490-G2), and a Brown potentiometer recorder, modified to operate as a servo-amplifier, were all tested experimentally. The General Electric unit was used routinely for economic reasons. However, the Brown servo-amplifier is recommended for over-all operational and stability characteristics.

Typical Compensator. One particular apparatus for iR compensation based upon the outlined scheme was designed to occupy a relatively small space beside the polarograph (Figure 2). The amplifier in this compact unit was a modified General Electric self-balancing potentiometer, having a gain of 101. A 1000-ohm 10-turn potentiometer served as the resistance, R_1. Thus, the range of the instrument extended to 100,000 ohms cell resistance. The output of the amplifier was connected in series with the cell, and polarized so that it acted in opposition to the iR drop and added to the applied potential. A voltmeter connected across the output of the amplifier continuously indicated the compensating voltage. The voltmeter used had full scale ranges of 0 to 0.25 and 0 to 1 volt. Performance of the compensator could be checked at any time during operation by comparing the meter reading with the calculated iR drop. The details of construction of the voltmeter-potentiometer unit in terms of terminals, switches, pilot light, etc., are evident from the photograph.

Automatically corrected polarograms were recorded directly with this unit (Figure 3). Accurate iR compensation and easily measured current-voltage waves were obtained in all cases.

Table I. Experimental Conditions Used in Evaluating *iR* Compensator

Sample	Phenol	*m*-Dinitrobenzene	*N,N'*-di-*sec*-butyl-*p*-phenylenediamine	Oxygen
Solvent	50% water–50% isopropyl alcohol	5% water–95% ethyl alcohol	50% isopropyl alcohol–50% iso-octane	Isopropyl alcohol
Electrolyte	0.5*M* NaOAc, acidified to pH 5.2 with HOAc	0.1*M* NH_4Cl	0.1*M* LiCl	0.2*M* LiCl
Type of electrode reaction	Oxidation	Reduction	Oxidation	Reduction
Indicating electrode	Graphite	Dropping mercury	Wax-impregnated graphite	Dropping mercury
Approx. cell resistance, ohms	400	2000	9000 and 19,000	10,000
Maximum *iR* correction, volts	0.02	0.07	0.19	0.28
Polarization rate, mv. per sec.	1.24	1.86	1.24	2.48
Literature reference	(*3*)	(*6*)	(*2*)	

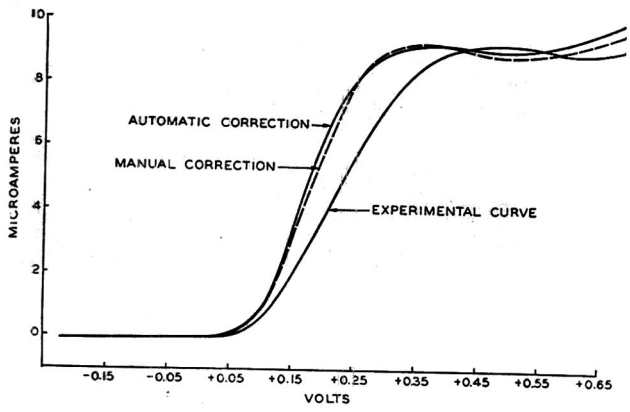

Figure 4. Comparison of manual and automatically corrected polarograms

2.5 ml. of dibutylphenylenediamine in 100 ml. 1 to 1 iso-octane–isopropyl alcohol, 0.1*M* in LiCl
Wax-impregnated graphite electrode *vs.* Ag-AgCl cathode
1.24 ml. per second; stirred test solution

However, the General Electric amplifier is not recommended for several reasons, one of which is the noise obtained at high current levels. Erratic oscillations on the compensated limiting plateau (Figure 3) may have been due to one or more of several factors and the exact cause was not determined. The recommended Brown potentiometer unit offers considerable advantage in this respect; barely detectable noise levels were obtained when using this amplifier.

Operation. The compensator is inserted in the circuit in series between the polarograph and the reference electrode. After the unit has been allowed to warm up for 1 minute, during which period the polarographic cell resistance can be measured, the amplifier and the voltmeter zero readings are adjusted and the calibrated potentiometer is set in accordance with the measured cell resistance. A corrected polarogram can then be recorded directly.

EVALUATION OF COMPENSATOR

Performance of the compensator was checked under conditions typical of those normally encountered in polarographic work. Reproducibility was determined from a series of measurements made under identical conditions. Results obtained with the automatic compensator were compared to those obtained conventionally by using calculated corrections for *iR* drop.

The specific conditions used in evaluation of the compensator are listed in Table I. All cell resistances were measured with a 60-cycle alternating current conductivity bridge. Solvents and electrolytes were varied so that resistances ranged from 400 to 19,000 ohms; *iR* correction values ranged from 0.02 to 0.28 volt. Three electrodes—i.e., the dropping mercury, graphite, and wax-impregnated graphite electrodes—were used to verify applicability to both cathodic and anodic polarography as well as to ascertain the effect of electrode nature. Polarization rates were also varied.

Automatically and manually corrected polarograms were compared as shown in Figure 4. Operation of the compensator was satisfactory over the entire oxidation wave of *N,N'*-di-*sec*-butyl-*p*-phenylenediamine, using the wax impregnated electrode. Differences between the automatically and manually corrected polarograms never exceeded 10 mv. Experimental values from multiple determinations with and without the compensator are tabulated in Table II. Average half-wave potentials were found to be identical within the limits of error; the difference between standard deviations of 0.009 and 0.007 volt has no statistical significance. Limiting current values for the automatically corrected polarograms were slightly lower than those determined by manual correction, although the average values of 3.64 and 3.88 μa. do not differ statistically. However, reduced residual currents were always observed when automatic correction was used, indicating that the compensator does cause a slight depression of current. Reproducibility was not adversely affected, as can be seen from the identical standard deviations for both methods.

Similar comparisons were made on the first reduction wave of dinitrobenzene, using the dropping mercury electrode (Table III). Average values for $E_{1/2}$ did not differ significantly; standard deviations were identical. Current values were within the limits of experimental error; the standard deviations of 1 and 2% are comparable to those normally obtained with the dropping mercury electrode.

Over-all results indicate that the compensator performs satisfactorily with either electrode under the conditions studied, and has no appreciable effect on either the value or reproducibility of $E_{1/2}$ and i_d measurements.

The compensator was also checked under other conditions possible with commercially available polarographs. Maximum damping provided by the Sargent Model XXI caused no difficulties. $E_{1/2}$ and i_d values calculated from automatically recorded polarograms did not differ significantly from those

Table II. Comparison of Automatic and Manual *iR* Correction with Wax-Impregnated Graphite Electrode

(Dibutylphenylenediamine in 1 to 1 iso-octane–isopropyl alcohol, 1.24 mv. per sec., stirred test solution)

	$E_{1/2}$, volt		i_d[a]	
	Automatic	Manual	Automatic	Manual
	0.171	0.174	3.36	3.56
	0.178	0.175	3.38	3.78
	0.156	0.165	3.82	4.26
	0.179	0.173	3.88	3.74
	0.168	0.174	3.66	3.74
	0.183	0.186	3.78	4.22
Av.	0.173	0.175	3.64	3.88
Std. dev.	0.009	0.007	7%	7%

[a] Microamperes per mg. in 100 ml. of test solution.

Table III. Comparison of Automatic and Manual *iR* Correction with Dropping Mercury Electrode

(1.0*M* *m*-dinitrobenzene in 95% ethyl alcohol; 1.86 mv. per sec.)

	$E_{1/2}$, volt		i_d, microamperes	
	Automatic	Manual	Automatic	Manual
	−0.502	−0.500	11.9	12.7
	−0.489	−0.509	12.4	12.4
	−0.498	−0.508	12.3	12.6
	−0.487	−0.496	12.6	12.3
Av.	−0.494	−0.503	12.3	12.5
Std. dev.	0.008	0.008	2%	1%

obtained by manual correction (Table IV). The effect of different polarization rates was also investigated; the data of Tables II, III, and IV were obtained using polarization rates of 1.24, 1.86, and 2.48 mv. per second, respectively; values calculated from automatically compensated polarograms were acceptable in all cases.

DISCUSSION

Two minor problems were encountered in the operation of the compensator. In following the oxidation wave of phenol in a solution of very low resistance (Table I), large current oscillations were recorded when the compensator was employed. Normally, no oscillations are observed with the graphite electrode. The apparent instability of the compensator as indicated by the oscillations was probably due to the high sensitivity of the amplifier. Consequently, the compensator should not be operated with low resistance media. This limitation is of little importance, because *iR* corrections are usually negligible under the latter condition.

The second problem was encountered only with the dropping mercury cathode. When this was used, voltmeter readings during operation indicated that consistently high correction values were apparently being applied. However, corresponding errors in the recorded polarogram could not be detected. The incorrect meter readings could have been due to its failure to follow rapidly changing currents. Because the meter serves only as an indicating device, operation of the compensator is unaffected.

Several distinct advantages result from the use of the compensator. First, by eliminating time-consuming manual corrections, polarographic calculation time is greatly reduced and immediate interpretation of polarograms is made possible.

Table IV. Effect of Damping on Performance of Compensator

(Oxygen in isopropyl alcohol containing 0.2*M* LiCl and saturated with air; 2.48 mv. per sec.)

iR Correction	Damping	$E_{1/2}$, Volt	i_d, μa.
Automatic	None	−0.362	25.0
Manual	None	−0.364	24.1
Automatic	Maximum[a]	−0.363	20.8
Manual	Maximum[a]	−0.365	22.1

[a] Damping position 2 on Sargent Model XXI Polarograph.

Secondly, the device is of particular value in anodic polarography employing solid electrodes. For example, both half-wave potential and limiting current values measured with graphite electrodes are greatly influenced by the rate at which potential is applied. Large cell resistances cause fluctuations in the polarization rate which, in turn, cause errors in quantitative measurements of half-wave potentials and diffusion currents. The errors are completely eliminated by use of the automatic compensator.

A third advantage is found in the interpretation of exploratory work, where unexpectedly high *iR* drops sometimes result in incomplete voltage spans when handling solutions of high resistance. In such cases, polarograms have to be repeated. With the compensator, the effective cell potential is always equal to the full range applied by the polarographic circuit.

Another advantage may be gained in the analysis of organic groups which are difficult to reduce. A resistance of 5000 to 50,000 ohms may be readily encountered with cells used in polarography when handling organic solutions. A current flow of 10 to 50 μa. would then result in an *iR* drop which, in many cases, could make it impossible to observe the more negative waves due to the lack of a sufficiently large potential at the cathode. The compensator eliminates this difficulty because the *iR* drop of the cell is canceled.

An alternative approach to the problem of high cell resistance would be the manipulation of solution composition and electrode characteristics so as to obtain low currents—i.e., currents of such magnitude that a correction for the *iR* drop in the cell need not be made. This would generally require a polarograph of high sensitivity, not commercially available, of the type described by Kelley and Miller (*5*). Such an approach would be useful when excess dilution of the sample is either necessary or desirable. However, in many cases, the added dilutions required to avoid *iR* corrections constitute an extra source of error, are time-consuming, and may be inconvenient. In the analysis of mixtures of two or more polarographically active components, present in dissimilar amounts, the currents may be of such magnitude as to preclude use of a sensitive polarograph. The *iR* compensator which has been described can be used with any type of polarograph and provides a more practical approach to problems involved in the more common analytical procedures.

If the resistance of the current-measuring resistor found in the polarograph itself is very high (over 10,000 ohms), such that the *iR* drop in it is appreciable, a suitable correction must be made. In the case of instruments such as the Sargent Model XXI Polarograph which employ a 2.5- or 5-mv. recorder, no correction is necessary, because the *iR* drop in the current-measuring resistor obviously cannot exceed 2.5 or 5 mv. without driving the recorder pen off scale. Such a correction is made automatically when a current amplifier recording arrangement is employed.

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Type Carbon Atom Analysis of Heavy Hydrocarbon Products—Correction

In the article on "Type Carbon Atom Analysis of Heavy Hydrocarbon Products" [ANAL. CHEM. 27, 1947–56 (1955)] on page 1949 Equation 1 should read:

$$Q_O = \frac{M_0(H_1 - H_0)}{2.016\ (100 - H_1)}$$

On page 1953, Table VII, Example 2, the boiling range should read: 166 at 760 mm. to 284 at 6 mm. of mercury.

SIMON MIRON

Microscopic Examination of Modified Starches

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Various microscopic techniques are useful for characterizing commercial modified starches. Granule aggregation can be detected by examination in water and glycerol media. The composition of starch blends can frequently be evaluated by granule counts in a haemacytometer. The species of a pregelatinized starch is determined by destroying the gelatinized material with enzyme and examining the ungelatinized residue. The Kofler microscope hot stage provides a simple and accurate measurement of gelatinization temperature, particularly useful for studying the effects of various adjuncts on starch gelatinization. Chemical modification of granular starches progressively lowers the gelatinization temperature, and the extent and uniformity of derivatization can be estimated by this means. Positively charged dyes—e.g., methylene blue—stain anionic products such as oxidized starches, phosphate esters, and carboxymethyl ethers. Cationic starches stain only with negatively charged dyes—e.g., light green SF. Intensity and uniformity of staining provide evidence of blended starches and nonuniform derivatives.

THE microscope has been traditionally employed for identifying the species of an unknown starch from the size and shape of its granules. Newer microexamination techniques have proved invaluable for the diagnosis of process engineering problems, for the analytical characterization of modified starches, and as an important aid in general starch research. A brief inspection under the microscope frequently provides information which could not possibly be obtained by any other means.

The present studies employed a polarizing microscope fitted with Ahrens prisms and capable of magnifications of 100 to 500 diameters. Individual objectives should be centerable and of the "quick-change" type. A highly useful accessory is the Kofler micro hot stage, originally designed for melting point determinations and microsublimations, but ideally suited for determining gelatinization temperatures of starches.

PRELIMINARY EXAMINATION

Initial inspection of a starch should be made in water suspension, never on the dry granules. A 0.2 to 0.3% suspension gives about the proper population of the field. The species of starch, judged by the size and shape of the granules and the position and fissuring of the hilum, can then be determined by comparison of the unknown against samples of known starches, preferably on the same slide. The procedure of alternate inspection of the unknown against the known frequently brings out minor differences which cannot be derived from comparison with a photomicrograph, although there are many good ones in the literature (*6, 8*). A collection of permanent slides of the various starches mounted in Canada balsam or in one of the polymerizing synthetic resins is convenient for this purpose. The position of the hilum in the granule, whether centric or eccentric, is an important aid in identifying the starch species. With certain starches the hilum is difficult to discern; the eccentricity of the interference cross under polarized light then offers the best criterion of its location. The relative brightness of the polarization cross is also frequently helpful for identification.

The starch should likewise be examined in glycerol medium, primarily to provide information on granule aggregation. Starches may sometimes be dried under too rigorous conditions of heat and moisture, producing a slight gelatinization of the surface of the granule; as a consequence, the granules will adhere together in clumps which do not break apart in glycerol medium. Figure 1 depicts a rice starch properly dried so that each granule is separate. In contrast, Figure 2 shows a second rice starch in which the granules are clumped into large aggregates. This product was dried in a flash dryer, and the clumping was provoked by excessive moisture of the starch feed to the dryer, together with too high an inlet air temperature. Flash drying is usually satisfactory for starch, but operations should be checked occasionally with the microscope. Granule aggregation has several undesirable consequences. The bulk density of the dry starch is substantially reduced—for example, the density of the aggregated rice starch in Figure 2 was 26 pounds per cubic foot compared with 33 pounds for the disaggregated sample in Figure 1. In addition, aggregated starch is definitely inferior for such uses as cosmetic dusting powder or as a release agent for automobile tire molds. In most cases, these aggregates fall apart immediately and completely when the starch is suspended in water rather than in glycerol. Any aggregation which persists in water medium may cause trouble in subsequent use, sometimes gelatinizing to give micro lumps which do not break down on cooking. An instance is the clay coating of paper, where unmodified starch is pasted and converted with liquefying enzyme to provide a carrier and adhesive for the clay. There have been isolated instances where granule aggregates have persisted through this process, subsequently giving specks on the high-gloss paper and interfering with printing. Hence, examination of the starch in both glycerol and water media offers a useful test for quality control.

Nonaqueous mountants will sometimes reveal the presence of adjuncts. For example, a commercial mixture of corn dextrin and carboxymethylcellulose (for use as a rayon warp size) was readily identified when examined in glycerol medium under polarized light, which showed the birefringent fibrils of the cellulose derivative. Similarly, the presence of admixed borax

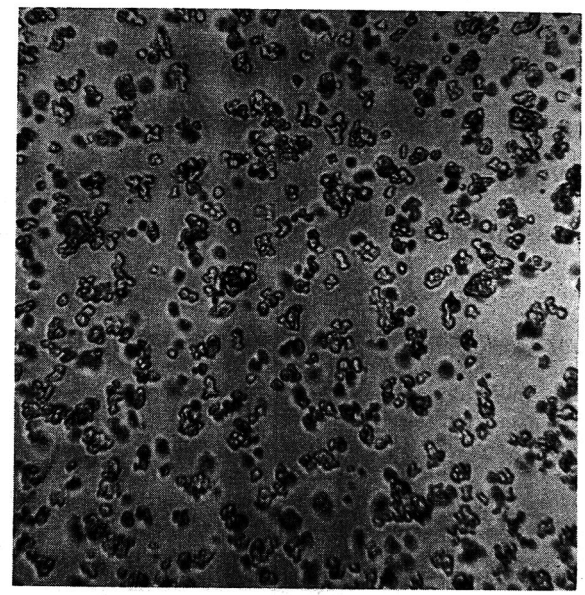

Figure 1. Disaggregated rice starch, mounted in glycerol

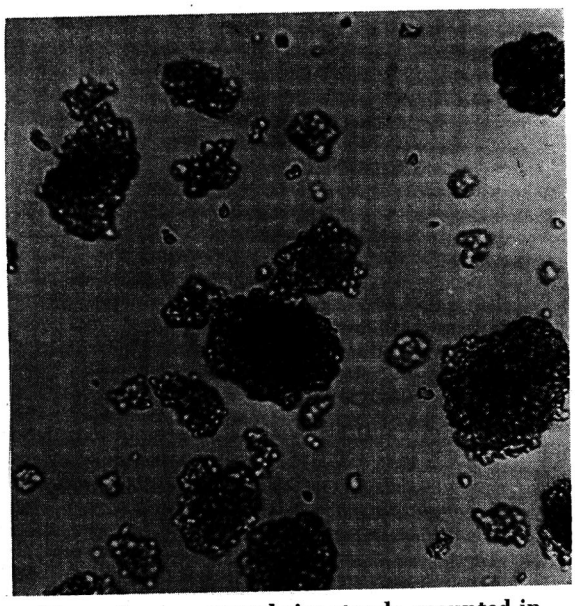

Figure 2. Aggregated rice starch, mounted in glycerol

or boric acid can be discerned by mounting in light mineral oil. Fissures and hilum damage are readily detected by mounting in clove oil, which has the same refractive index (1.530) as starch, thus making the hilum stand out in sharp contrast.

GRANULE COUNTS

It is frequently necessary to estimate the proportion of two different types of starch granules in a sample. This can be done with a blood-counting cell or hemacytometer, preferably of the "bright-line" type with Neubauer double rulings. For the estimation of waxy starches, an aqueous suspension of the sample is stained lightly and uniformly with iodine and the red-staining waxy granules and the blue-staining normal granules are then counted. All waxy starches (except genetically pure preparations) contain a small percentage of blue-staining starch granules due to cross pollination. For quality control over waxy starch production, accurate counts on 500 to 600 granules can be made within 10 minutes by means of a hand tally. Precision is ±1 part in 10, adequate for routine control. Commercial blends of waxy maize and normal corn starch are now common and their identity and composition can also be readily established by this method.

Mixtures of two different starch species are more difficult to determine by count because of difference in granule size. In such cases, the unknown sample must be compared against known synthetic mixtures. For example, mixtures of Idaho potato starch and corn starch, made up to contain 10, 25, 50, and 75% by weight of corn starch, were observed to contain 49, 70, 84, and 94%, respectively, by number count of corn starch granules.

Granule counts may also be used to estimate minor proportions of partially swollen granules in process starch samples. Such gelatinization may be caused in an aqueous starch slurry by the incautious addition of agents such as alkali, or by local overheating, such as might be caused by contact with the wall of a steam-jacketed kettle. The presence of partially swollen granules may cause such processing difficulties as plugged filters, slow settling, and excessive solubles, as well as indispersible grit in the finished dry starch. The literature makes frequent mention of Congo red as a preferential stain for partially swollen granules. However, diminution or absence of the characteristic interference cross when viewed under polarized light is a much more positive and readily discernible criterion of swollen granules. In most instances of this sort, the partially swollen granules are sharply defined and may be easily and accurately counted in the hemacytometer.

GRANULE SIZE DISTRIBUTION

During studies on size fractionation of starch granules, it was necessary to obtain granule size distribution curves for corn and sorghum starches. To do this, photomicrographs were taken of a number of representative starch samples in water medium, and these were enlarged to exactly 500 diameters. Granule diameters were then measured manually with a rule to the nearest millimeter—i.e., equivalent to 2 microns. It was found necessary to measure 600 to 1000 granules of each starch sample to obtain reproducible curves. Figure 3 shows the granule size distribution of these starches on a population basis. The "number average" granule diameters for commercial corn and sorghum starches were 9.2 and 15.0 microns, respectively, as calculated by the formula

$$\text{Number average diameter} = \frac{\Sigma\,(\text{number \% of each granule size} \times \text{granule diameter})}{100}$$

These two starches cannot be definitely distinguished by visual inspection, because this difference in average size cannot be readily detected by the eye.

From a practical standpoint, size distribution curves are significant only when converted to a weight percentage basis. Assuming that all granules have the same density, this calculation was made by multiplying the number percentage of each granule size by the cube of its diameter.

$$\text{Weight \%} = \frac{\text{number \% of granules of specified diameter} \times \text{cube of diameter} \times 100}{\Sigma\,(\text{number \%} \times \text{cube of diameter})}$$

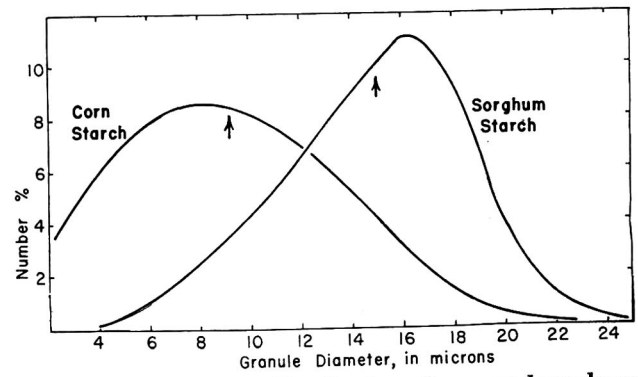

Figure 3. Granule size distribution of corn and sorghum starches, calculated on a population basis

Arrows indicate respective "number average granule diameters"

Figure 4 shows the granule size distribution of the same corn and sorghum starches calculated on a weight percentage basis. For example, a value of "8 weight %" of granules of 17-micron diameter would signify that 8% by weight of the total starch has a granule diameter between 16.5 and 17.5 microns. The "weight average granule diameters"—i.e., the diameter of a granule of average weight—for corn and sorghum starches are 14.1 and 17.4 microns, respectively, as calculated by the formula

$$\frac{\Sigma\,(\text{weight \% of each granule size} \times \text{granule diameter})}{100}$$

Such data have utility in following the recovery of starch in tabling and centrifuging operations. As a further example, it might be desired to separate that fraction of corn starch having a granule diameter of 8 microns or less. This fraction consti-

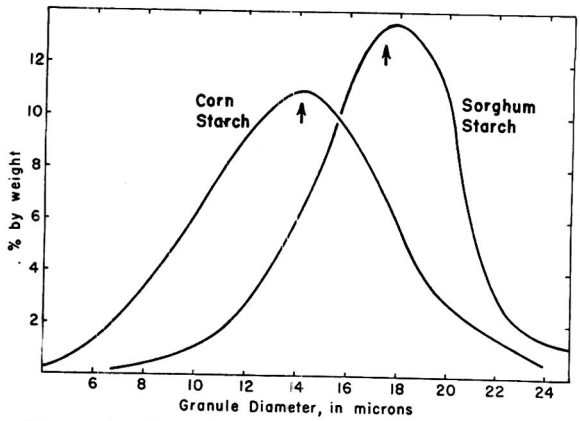

Figure 4. Granule size distribution of corn and sorghum starches, calculated on a weight percentage basis

Arrows indicate respective "weight average granule diameters"

tutes 45% of the total number of granules, but it represents less than 8% by weight of the total starch.

PREGELATINIZED STARCHES

A variety of pregelatinized starches are manufactured by drying a precooked starch paste on drum dryers, on heated squeeze rolls, or in a spray dryer. These products are marketed for such purposes as paper beater size, foundry core binders, and as a bodying agent for oil-well drilling muds. The method which is used for drying is usually determined by suspending a sample in glycerol and examining it under the microscope. If the particles appear as irregular chunks, the pasted starch was probably dried in a relatively thick layer on a drum dryer, then ground and screened. If the starch is in the form of thin irregular plates, like shattered window glass, drying was effected on the squeeze rolls. Spray drying gives hollow spheres enclosing an air cell.

It is frequently necessary to identify the species of starch in such pregelatinized products. Preliminary examination should be made in water suspension under the polarizing microscope. If the product has been insufficiently pasted, it will show a multitude of ungelatinized granules with their characteristic polarization crosses. In such instances, the species of starch can be readily identified. An approximation of the proportion of ungelatinized starch can be made by comparison against synthetic blends of granular and pregelatinized starches. Commercial products have been encountered in which as much as 20 to 30% of the starch remained ungelatinized and, hence, ineffective for the contemplated use. Usually the amount is less than 1%, and frequently it is almost vanishing. These latter products present difficulties in identification, since the few remaining granules are obscured by the mass of gelatinized flakes. In these cases, 5 to 20 grams of the starch sample is stirred into 100 ml. of a filtered solution of a low-temperature amylase, such as Rhozyme S or Vanzyme. The enzyme solution must first be carefully filtered to remove any granular starch which may be used as a carrier for the enzyme. The mixture is maintained at 40° C. for 30 to 60 minutes to digest and dissolve the gelatinized starch flakes, leaving the ungelatinized granules intact. The solution is then centrifuged, the supernatant liquid is decanted, and the trace of insoluble residue examined under the polarizing microscope. No commercial pregelatinized starch has yet been encountered which was not identifiable by this means. Apparently, even thoroughly cooked products contain a few thousandths of a per cent of intact granules which somehow escape gelatinization. Because of its sensitivity, the method must be used with considerable care and discrimination. In the laboratory of a starch processing plant, there are always enough air-borne starch granules to represent a significant source of contamination. If corn and potato starch granules are detected in a sample, some question may arise whether this represents a blend, or whether contamination has occurred during manufacture. Considerable difficulty is encountered with high-protein corn and wheat flours, where protein obscures the ungelatinized granules. In this connection, when the honeycomb structures of cell walls are observed in a pregelatinized high-protein corn sample, an origin of dry-milled corn is presumed.

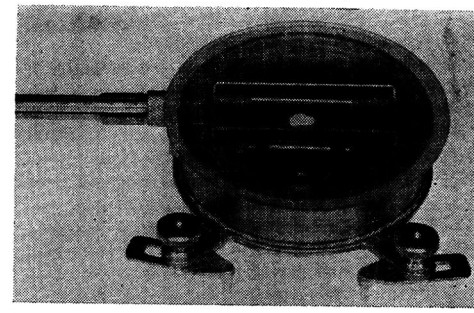

Figure 5. Kofler microscope hot stage for determination of gelatinization temperatures

This enzyme digestion technique sometimes has usefulness in identifying the starch species employed in surface-sizing a fabric. A strip of the fabric is suspended in a warm enzyme solution for 15 to 30 minutes. If the strip is then agitated, a perceptible amount of material will slough off the surface. The strip is removed and thoroughly rinsed with a jet from the wash bottle. The combined enzyme solution and rinsings are centrifuged, and any slight trace of precipitated material is examined under the microscope. Persistent granules can frequently be identified, particularly if a thick-boiling starch has been employed in sizing.

DETERMINATION OF GELATINIZATION TEMPERATURE

The gelatinization temperature is best defined as the point at which the starch granules lose their polarization crosses when heated in a swelling medium. This loss of polarization immediately precedes swelling. Actually, all the granules in a given sample do not lose their polarization simultaneously, but usually over a range of some 8° to 10° C. Hence, a proper measurement of gelatinization temperature should indicate both the initial and terminal points. Determination of gelatinization temperature by the usual hot water bath technique is tedious and very frequently inaccurate. Even with slow heating in a triple bath, hot walls cause local gelatinization of the granules. However, it has been found that the Kofler electrically heated microscope stage (Arthur H. Thomas Co., Philadelphia) provides a quick, simple and accurate evaluation of gelatinization temperature. The Kofler stage (Figure 5) consists of a circular metal block heated by internal resistance coils. On this block is placed a glass slide carrying a droplet of starch suspension. To ensure uniformity of temperature, a glass baffle is placed above the slide, and the whole assembly is covered with a close-fitting glass plate. A thermometer is inserted in the metal block in such a position that the stem can be kept under constant observation. A small variable transformer provides accurate temperature control. The hot stage should be tested by determining the melting points of known substances.

To determine the gelatinization range, the starch is slurried in water to give a 0.1 to 0.2% suspension. A small drop of this suspension is then spotted on a microscope slide. The drop is surrounded by a continuous ring of high viscosity mineral oil and a cover glass dropped on in such a way that the aqueous drop is completely enclosed by an oil barrier, with no air bubbles under the cover glass. The purpose of this oil barrier is twofold: (1) to prevent the escape of steam which fogs the glass baffle, and (2) to prevent air channels from penetrating under the cover glass and disturbing the field. The rate of temperature rise

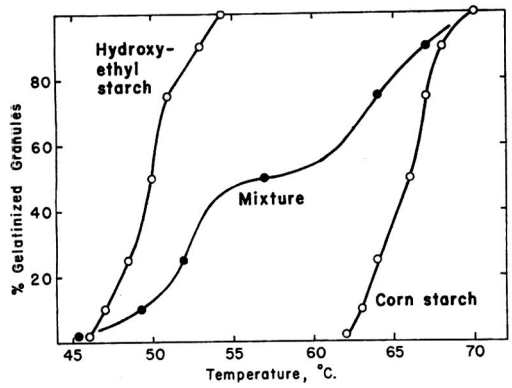

Figure 6. Gelatinization curves for corn starch, for hydroxyethyl corn starch (0.12 degree of substitution), and for a 50–50 mixture of these two starches

Latter sample showed no granules gelatinizing in range of 55° to 60° C.

when approaching and during the gelatinization range should be about 2° C. per minute, corresponding to the 23-volt setting on the variable transformer. The field is continuously watched under normal lighting to determin ethe temperature at which the first three or four granules show a change in physical outline. Loss of polarization crosses in these granules can be immediately confirmed by introducing the Ahrens analyzer. This is the initial point in the gelatinization range. Heating is continued, and the field is now watched under polarized light. When the polarization crosses have disappeared in all but perhaps two or three granules in the entire field, this temperature is recorded as the completion of the gelatinization range. Frequently, a very few granules will persist for an additional 5° C. or more, but it seems advisable to neglect these. Duplicate or triplicate runs should be made and the results averaged; in general, precision has been ±0.5° C. It is often advantageous to include a midpoint in the gelatinization range—namely, the temperature at which approximately half the granules have lost their polarization crosses. In certain of the present studies, full gelatinization curves have been obtained by observing the temperatures at which the following percentages of granules have lost birefringence: initiation, 10, 25, 50, 75, 90%, completion. These points can be determined accurately by visual inspection during the gelatinization, and this technique gives much more reproducible curves than the water bath method, despite the fact that no exact count of gelatinized granules can be made. Typical sigmoid gelatinization curves are illustrated in Figure 6.

The various species of starch have different gelatinization temperatures, which are fairly uniform within any given variety.

Corn starch, various batches	62–67–70° C.
	62–73° C.
	62–71.5° C.
Waxy maize starch	62.5–68–72° C.
Sorghum starch, various batches	68–75° C.
	69–74° C.
	68–74.5° C.
	68.5–75° C.
	68.5–75.5° C.
Waxy sorghum starch	67.5–70–74° C.
Idaho potato starch	56–67° C.
Maine potato starch	59–62.5–67.5° C.
Dominican tapioca	58.5–64.5–70° C.

These represent temperatures which cannot be approached too closely in the starch factory without risking partial gelatinization. The waxy starches have substantially the same gelatinization temperatures as their normal blue-staining counterparts. Hence, the presence of the linear starch fraction does not affect initiation of granule swelling, though it is recognized that waxy granules swell more rapidly and are much more fragile than the normal blue-staining starches.

The gelatinization range becomes an important consideration when starch is used in other than simple water systems. For example, increasing concentrations of ammonium nitrate or urea progressively lower the gelatinization temperature, while ammonium sulfate markedly raises it.

Corn starch, in water	62–73° C.
Same, in NH_4NO_3 solution	
10%	62–75° C.
20%	54.5–70.5° C.
30%	52–66.5° C.
40%	44.5–63° C.
50%	42–61° C.
60%	Room temp.–57° C.
70%	Room temp.–55° C.
80%	Room temp.–53° C.
Same, in urea solution	
10%	51–64° C.
20%	39–55° C.
30%	Room temp.–47° C.
40%	Complete at room temp.
Same, in $(NH_4)_2SO_4$ solution	
10%	78–90° C.
20%	88–99° C.
30%	No gelatinization on boiling

Esterification or etherification of granular starch progressively lowers the gelatinization temperature as the degree of substitution (D.S.) is increased (*3*). This is exemplified by the gelatinization ranges of several hydroxyethyl ethers and of the "Feculose" acetate esters (*9*) of corn starch.

Feculose starch acetate, 0.04 D.S.	56–58–63° C.
0.08 D.S.	48–52–56° C.
0.12 D.S.	41–46–51° C.
Hydroxyethyl corn starch, 0.04 D.S.	57.5–67.5° C.
0.12 D.S.	45.5–54.5° C.
Unmodified corn starch	62–67–70° C.

In this way, the gelatinization temperature provides evidence of the uniformity of substitution of a starch ester or ether, since any granules which remain unswollen at 68° to 70° C. necessarily cannot be substituted to any significant extent. Frequently, this can be traced to inadequate mixing of the reactants—e.g., alkali and ethylene oxide—leaving pockets of unreacted starch. A qualitative estimate of the uniformity of substitution can be had by plotting the full gelatinization curve (Figure 6). Thus a uniformly substituted hydroxyethyl starch (0.12 D.S.) gives a sigmoid gelatinization curve similar to that of the parent corn starch, but at a substantially lower temperature. An artificial 50–50 mixture of this hydroxyethyl starch with unmodified corn starch gives the composite curve as shown; such a curve for a commercial product would immediately indicate either a blend or a nonuniform derivative.

When a normal starch granule is progressively heated on the hot stage above its gelatinization temperature, it continues to swell until its outlines become indistinct. However, if a starch granule swells only two to three times its original diameter and then persists without further change despite rising temperature, the inference is that it has been chemically cross-bonded—e.g., by epichlorohydrin to give ether bridges, or by phosphorus oxychloride to give ester cross linkages. A corn starch cross-bonded with epichlorohydrin had a gelatinization range of 64° to 74° C., not significantly different from the parent starch. When heated on the hot stage, this product swelled in normal fashion until a temperature of 80° C. was reached; thereafter it showed no further swelling when heated to higher temperatures.

Gelatinization temperature likewise has application to certain phases of fundamental starch chemistry. For example, the starches from immature dent corn (supplied by courtesy of the Northern Utilization Research Branch, U. S. Department of Agriculture) showed low gelatinization temperatures, increasing with maturity.

Days after Pollination	Gelatinization Temperature, ° C.
21	50–57.5–61
35	51.5–68–73
Mature	58–67.5–74

The immature starches are, of course, smaller in granule size. However, this does not explain the lower gelatinization temperature, because the small-granule fraction (less than 8 microns) isolated from commercial corn starch had the same gelatinization range as the parent starch. It may be that the extent of molecular association within the granule increases during maturing of the corn and causes a rise in gelatinization temperature.

Similarly, the so-called heat-moisture treatment reported by Sair and Fetzer (*7*) apparently increases the micellar network within the granule. When granular potato starch is carefully steamed for several hours in an autoclave, the granules suffer no visible change in physical form or optical properties. However, the x-ray diffraction pattern is altered from a B spectrum to an A spectrum. Also, the treated potato starch gives "short" opaque pastes which set up to rigid gels on cooling, closely resembling corn starch in its behavior. The Kofler gelatinization temperature is raised 5° to 10° C., and the granules exhibit a restricted swelling similar to the chemically cross-bonded starches. These observations suggest that the physical differences between corn and potato starches may be due as much to different levels of micellar organization within the granules as to the content and molecular weight of their linear fractions.

IDENTIFICATION OF IONIZED STARCHES BY DYE ADSORPTION

Starches oxidized by hypochlorite absorb methylene blue, a fact which has suggested a microscopic staining test for identification of these products (*1*). However, the literature is confused on this subject, and no rational explanation has ever been offered for the staining reactions of various starches with different organic dyes (*5*). It now appears that these reactions are due entirely to the ionic charge on the starch; a negatively charged starch will stain only with positive or cationic dyes, and vice versa. Negativity may be imparted to a starch by such means as oxidation or carboxymethylation (to introduce carboxyl groups), or by phosphorylation (ionized starch phosphate). A starch may become cationic by introduction of positively charged substituted ammonia groups, and consequently stain only with anionic dyes. A neutral or electrically balanced starch will not stain with any dyestuff, and conversely a charged starch will not stain with an electrically neutral dye.

The following biological stains (identified by Colour Index numbers) have been satisfactorily used for the examination of starches:

Positively charged dyes, staining negative starches. Methylene blue (C.I. 922), crystal violet (C.I. 681), malachite green (C.I. 657), methyl green (C.I. 685), safranine O (C.I. 841), thionine (C.I. 920), neutral red (C.I. 825)

Negatively charged dyes, staining positive starches. Light green SF yellowish (C.I. 670), acid fuchsin (C.I. 691), eosin Y (C.I. 768), orange G (C.I. 27)

Choice of a particular stain is largely a matter of personal preference; methylene blue and light green SF are usually employed in this laboratory.

To carry out the test, approximately 50 mg. of the powdered starch is placed in a test tube, 20 to 25 ml. of an aqueous 0.1% solution of the appropriate dye is added, and the mixture is allowed to stand for 5 to 10 minutes with occasional agitation. When a large number of samples is to be tested, the tubes may be stoppered, attached with rubber bands to a low-speed rotating shaft, and turned end over end for 5 minutes. The tubes are then allowed to stand until the starch is completely settled, and the supernatant dye solution is decanted. The starch is washed by repeated suspension and settling in distilled water, until the supernatant liquid is substantially colorless. These operations can be speeded up by centrifuging, particularly with small-granule or partially gelatinized starches which settle slowly. It is not possible to stain or wash too much, because the individual granule adsorbs and retains only its specific quota of dye. Understaining may occur if the dye solution becomes exhausted. Therefore, if the supernatant solution appears appreciably lightened in color, the starch should be allowed to settle, the liquid decanted, and fresh dye solution added to the starch. In general, the pH of the starch to be tested should be in the range of 4 to 7. Acidic or alkaline starches should be neutralized, then washed several times by decantation with distilled water before the addition of dye solution. The presence of salts interferes with the adsorption of dye, and starches with high salt content should be washed with distilled water. Gritty starches should first be soaked in water to break up persistent aggregates.

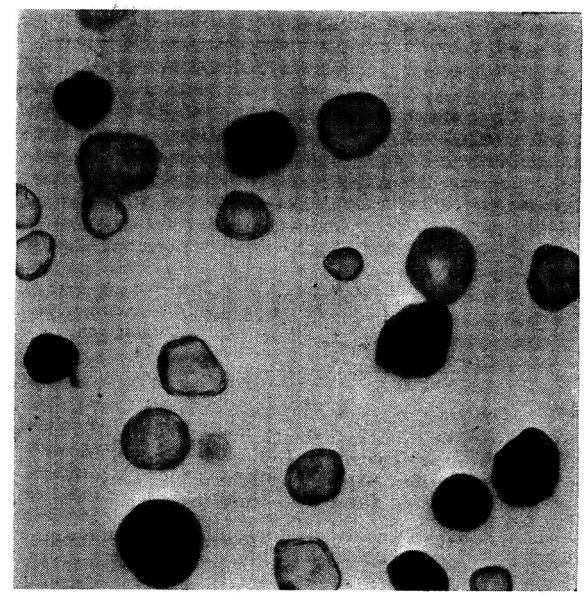

Figure 7. Mixture of equal parts of corn starch (light granules) and hypochlorite-oxidized corn starch (dark granules), stained with methylene blue

The intensity of staining is readily evaluated by examination under the microscope. The following tabulation shows the reactions of various common starch types.

Negative starches, staining with cationic dyes
- No staining. Cationic starches
- Faintly stained. Defatted corn starch, defatted wheat starch, corn torrefaction dextrins, tapioca starch, waxy maize starch
- Lightly stained. Corn starch, rice starch, wheat starch, thin-boiling acid-modified corn starches, white dextrins, hydroxyalkylated corn starches
- Moderately stained. Slightly oxidized starches
- Strongly stained. Potato starch (due to natural phosphate ester), thin-boiling oxidized starches, carboxymethyl starch (0.04 D.S.), sulfocarboxylic diesters, phosphorylated starches

Positive starches, staining with anionic dyes
- No staining. All negatively charged starches
- Moderate to strong staining. Various starches containing positively charged substituted ammonia groups

The intensity of staining indicates the electrical charge on the individual granule. For example, a starch oxidized with nitrogen dioxide (*2, 4*) and containing 9.3% carboxyl, stained several shades darker with any of the positive dyes than a similar product containing only 1.8% carboxyl; a cationic starch containing 0.52% amino nitrogen stained more intensely than a similar product containing only 0.11% nitrogen.

Starches which contain a linear fraction strongly absorb cationic or anionic surfactants, and subsequently stain in accordance with the ionic charge so imparted. For example, if corn starch is suspended in a dilute aqueous solution of sodium lauryl sulfonate, then filtered and thoroughly washed with water, the product stains strongly with methylene blue. When similarly treated with an aqueous solution of octadecyl trimethyl ammonium chloride, the same corn starch does not stain at all with methylene blue, but stains strongly with any of the negative dyes. Commercial corn starch stains slightly more than defatted corn starch because of the negativity imparted by the natural fatty acid. If the presence of such adsorbed material is suspected, the starch sample should be Soxhlet-extracted for 24 hours with 95% ethyl alcohol prior to staining tests.

Most flake-dried pregelatinized starches can be tested by these same techniques. When methylene blue is used to stain a dry blend of pregelatinized corn starch and pregelatinized carboxymethyl starch the corn starch stains a faint blue and the carboxymethyl starch, a deep blue. This difference is readily distinguishable under low power magnification—i.e., 50 to 100 ×. While rare in this country, such mixtures of different pregelatinized starches are frequently encountered in the European markets. Obviously, staining techniques are useless if the component starches are mixed before roll drying, since there is then no differentiation between the individual flakes. Difficulties are likewise encountered with products which tend to dissolve in cold water, such as a pregelatinized hydroxyethyl starch of high degree of substitution—e.g., 0.2 D.S. However, the more usual roll-dried corn, wheat, and potato starches give little trouble.

Several specific examples will further demonstrate the practical utility of these staining tests. Figure 7 shows a 50–50 mixture of unmodified corn starch and a thin-boiling hypochlorite-oxidized corn starch, stained with methylene blue. The two starches are sharply differentiated, and their relative proportions can readily be determined by granule count in the hemacytometer. The identity of this product as a deliberate blend could not possibly be determined by any other known method. Occasionally, oxidized starches are encountered in which the individual granules exhibit all levels from light to strong staining. This necessarily indicates nonuniform modification due to inadequate mixing of starch and oxidizing agent. Such products are more frequently encountered in dry or semidry conversions—e.g., by oxidation of dry starch with nitric oxide gas. Staining the starch granule does not interfere with its birefringence under polarized light. Hence, if any of the component starches in a mixture has undergone partial gelatinization, its identity can be immediately determined.

For demonstration purposes, a mixture of hypochlorite-oxidized starch, defatted corn starch, and cationic starch may be stained with a mixed solution of methylene blue and eosin Y. The granules stain blue, colorless, and red, respectively. Such a starch blend would not be feasible commercially, since the cationic and anionic starches would coprecipitate. Indeed, it is difficult to prepare a satisfactory microscope slide of the stained mixture, owing to the tendency of oppositely charged granules to aggregate into clumps.

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Ampoule Combustion–Isotope Dilution Technique for Organic Nitrogen

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A rapid, convenient, and precise means was needed for the quantitative determination of elemental nitrogen, especially where the available sample is very small, as in paper chromatography. The method described makes it possible to determine elemental nitrogen in organic compounds with a precision comparable to that achieved in the standard micro-Dumas determination but with sample sizes well under 1 mg. It is easily adaptable to routine operation.

IN THE ultramicro determination of nitrogen in various solid organic compounds, using the principle of isotope dilution, a fast, convenient, and compact technique is desirable for use on a routine basis for converting bound nitrogen into pure nitrogen gas and for introducing the tracing atom into the system under conditions of equilibrium. Grosse, Hindin, and Kirschenbaum (*2*) applied the isotope dilution–static combustion technique in the determination of elemental nitrogen, as well as carbon and oxygen, but their usual sample size was 20 to 40 mg. With samples of about 1 mg. or less the tracing element, along with the sample, must be introduced in solid form. It seemed that a technique in which the mixture could be burned in an individual sealed ampoule would be more readily adaptable to routine preparation of gas samples than the Dumas method (*4*), for example. Subsequent to the initiation of the investigations recounted in this and the following paper, Kirsten (*3*), in a brief scientific communication, described a sealed-tube combustion method for the determination of carbon, hydrogen, and nitrogen with 0.1- to 0.2-mg. specimens based upon gas volumetric measurements of the products of combustion. Kirsten gives no experimental data in his communication but merely remarks that his method "gave an accuracy somewhat less good than that of the Pregl method on a 5-mg. scale."

The method consists, essentially, of the following steps. The sample (tracer, or tracer and unknown) to be converted into nitrogen gas is sealed with an excess of cupric oxide in an evacuated ampoule of high-silica glass (Vycor) and heated in a furnace to complete combustion. The ampoule is opened in the previously evacuated ampoule breaker and the combustion gases, after absorption of carbon dioxide and water, are passed into a mass spectrometer where the isotope ratio of the nitrogen is determined.

APPARATUS AND REAGENTS

The ampoules are prepared from 9-mm. (outside diameter) 96% silica glass (*3*) tubing by closing one end of each 6-inch length in a hydrogen-oxygen flame. The ampoules are opened after combustion in the ampoule breaker shown in Figure 1. The breaker is constructed of brass, and the close-fitting plunger is

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well greased with a good vacuum grease to prevent leakage. It is sealed into its glass envelope with a vacuum wax such as Apieson wax W, and the glass envelope is joined into the top of the absorption tower with vacuum grease.

The ampoule breaker and absorption tower are shown assembled in Figure 2. The absorption tower is packed with a layer of Ascarite at the top and a layer of Dehydrite at the bottom, with glass wool separators. A mechanical pump and mercury diffusion pump are used to reduce the pressure to 1 micron or less before sealing; this is essential to reduce nitrogen contamination to a suitable level. Short lengths of carefully cleaned pressure tubing are suitable for making connection to the ampoules.

The Consolidated Nier mass spectrometer Model 21-201 was used for the isotope ratio measurements.

In working with such small amounts of sample it is essential that all sources of nitrogen contamination be avoided. Commercially available forms of reagent cupric oxide were found to contain too much nitrogen as impurity and consequently a special nitrogen-free form was prepared.

Satisfactory cupric oxide was prepared by passing dry electrolytic oxygen (USP grade) over electrolytic copper foil (0.005 inch thick) at 800° C. in a silica tube. The oxygen was dried by passing it successively through towers of pelletized sodium hydroxide, indicating anhydrous calcium sulfate, and Dehydrite. The foil, cut in strips about 0.25 inch wide, was heated until it crumbled, and the oxide was ground and sieved to pass 325-mesh, and reheated carefully to complete the conversion to cupric oxide.

The nitrogen content of this cupric oxide was found to be on the order of 0.0003%; some commercial samples (reagent grade) run as high as 0.05% in total nitrogen. This observation is at variance with the specification for reagent grade copper oxide, which allows 0.002% nitrogen. The ACS specification, however, determines nitrogen as ammonia and thus there is no necessity that the two methods agree.

Benzamide containing 62 atom % nitrogen-15 was prepared from ammonium nitrate of 62 atom % nitrogen-15 (in the ammonium group) by the reaction of ammonia gas with hot benzoic acid anhydride, Eastman Kodak Co. No. 557 red label. The ammonia gas was liberated by heating the ammonium nitrate with an excess of calcium oxide in an evacuated system, and pumped over the hot benzoic anhydride to complete the reaction. A slight excess (about 10%) of benzoic anhydride was used.

$$CaO + 2NH_4NO_3 \rightarrow Ca(NO_3)_2 + Ca(OH)_2 + 2NH_3$$

$$NH_3 + (C_6H_5CO)_2O \rightarrow C_6H_5CONH_2 + C_6H_5COOH$$

The product obtained was extracted with an excess of hot sodium bicarbonate solution and the insoluble benzoic anhydride filtered off. Benzamide crystallized from the chilled sodium bicarbonate solution. Final purification was effected by recrystallization from hot water, sublimation in vacuum at 117° C., and final recrystallization from hot water after filtration through a sintered-glass funnel.

Ordinary benzamide for dilution of the tracer was purified in the same manner. The final product in both cases was carefully dried under vacuum at 60° C.

Nicotinic acid (USP grade) was further purified by crystallization from hot water, sublimation in vacuum at 117° C., and final crystallizatiom from hot absolute ethyl alcohol, followed by filtration through a sintered-glass funnel. The final product was vacuum-dried at 100° C.

Nicotinamide (USP) was recrystallized from absolute ethyl alcohol and carefully dried. Caffeine (Kahlbaum) was used without further purification.

PROCEDURE

The ampoules are loaded with 100 mg. of cupric oxide, then outgassed by carefully preheating with a mild Bunsen burner flame while under vacuum and attached to the vacuum manifold previously described. Care must be taken not to decompose the cupric oxide appreciably; such decomposition is shown by dancing of the particles. Dry oxygen is allowed to enter the tubes after cooling. This procedure materially reduces the nitrogen blank obtained.

In the case of an isotope dilution analysis for nitrogen, both unknown and tracer must be accurately weighed and introduced into the ampoule to provide a total sample of about 3 mg. Small copper boats made from 0.005-inch electrolytic copper foil and weighing about 200 mg. were formerly used for this purpose, but were discarded when the copper foil was found to contribute substantially to the nitrogen blank. A platinum weighing stick or spatula is now used, the sample weights being determined by difference.

After the sample has been placed in the ampoule, the ampoule is constricted in a hydrogen-oxygen flame, attached to the vacuum manifold, pumped down to a few microns, and sealed. With volatile substances such as benzamide, the end of the ampoule containing the sample is kept cold during these operations by occasional immersion in a dry ice–acetone mixture.

The sealed ampoules are heated for 1 hour at 775° to 800° C. to complete the combustion. A temperature higher than 800° C. should be avoided because of frequent failure of ampoules; at

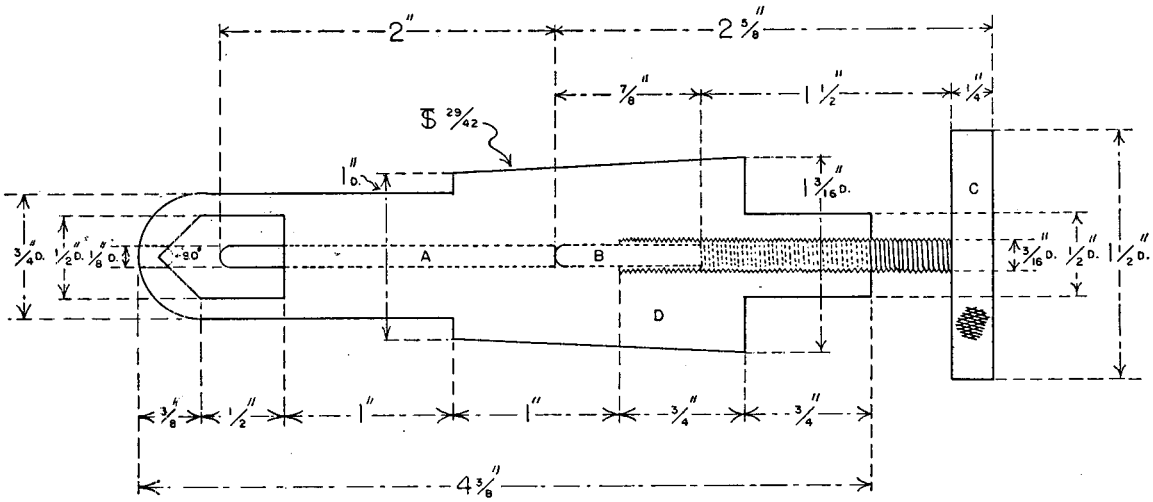

Figure 1. Top view of ampoule-breaking mechanism

Plunger *A* is carefully fitted and greased to prevent leakage
Body *D* may be made from aluminum to reduce weight

appreciably lower temperatures the combustion of some substances may not be complete. The ampoules are cooled slowly to allow reabsorption of excess oxygen by the copper oxide.

The cooled ampoule is placed in the ampoule opening assembly (Figure 2), which is connected to the mass spectrometer inlet system and thoroughly evacuated by a diffusion pump. The stopcock at the bottom of the absorption tower is then closed and the ampoule crushed. After a few minutes have been allowed for absorption of carbon dioxide and water by the Ascarite and Dehydrite, the nitrogen is allowed to enter the mass spectrometer inlet system, where the isotope ratio is measured. One or more passes of the gas back into the absorption tower may be advisable to assure complete removal of carbon dioxide and water. Carbon dioxide gives a mass peak at 28 due to CO^+ ions, which interferes with the nitrogen peak.

The nitrogen 29/28 ratio, corrected by multiplication of the measured ratio by the f factor of the mass spectrometer (*1*), is converted to atom per cent nitrogen-15 by the usual procedure.

$$\frac{100\,r}{2+r} - 0.38 = \text{atom \% excess } N^{15} = C_f$$

where r = corrected ratio $^{29}N_2/^{28}N_2$

In an isotope dilution analysis, the nitrogen in the unknown is calculated as follows:

$$X = \left(\frac{C_1}{C_f} - 1\right)\left(\frac{Y \times Z}{W}\right)\left(\frac{A_n}{A_y}\right)$$

where

W = weight of unknown sample
X = % nitrogen in unknown
Y = weight of tracer
Z = % nitrogen in tracer
C_1 = atom % excess N^{15} in tracer
C_f = atom % excess N^{15} in combustion mixture
A_n = atomic weight of nitrogen
A_y = mean atomic weight of nitrogen in tracer

Z must be the actual percentage of nitrogen in the tracer, not the percentage of nitrogen in the analogous nonisotopic compound.

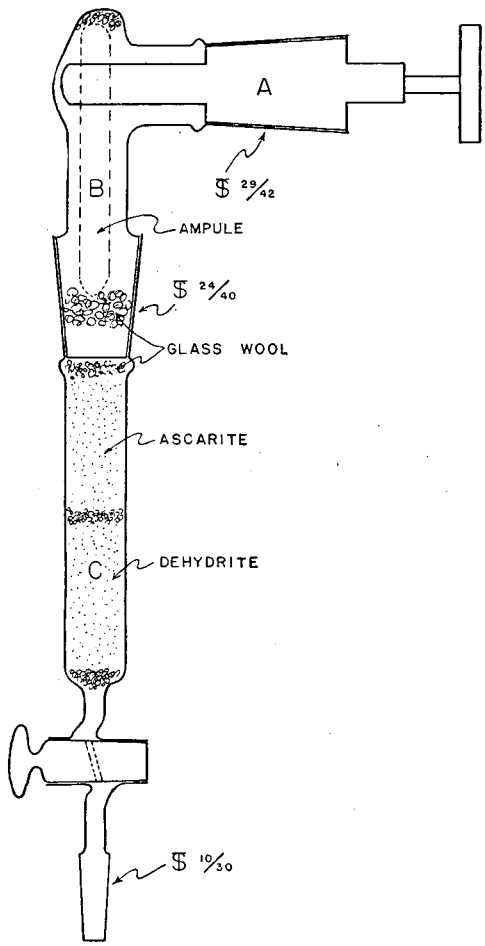

Figure 2. Ampoule opening assembly with ampoule breaker in place

B. **Ampoule in position to be opened**

Table I. Per Cent Nitrogen-15 Excess in Tracers

1:3 Dilution	1:1 Dilution
15.34	30.75
15.31	30.63
15.31	30.54
15.35	30.55
15.25	30.23
15.11	30.67
15.33	30.63
15.34	30.54
15.27	30.63
15.16	30.54
	30.55
Mean 15.28 ± 0.06	30.57 ± 0.09

RESULTS AND CONCLUSIONS

Benzamide tracer was prepared from the 62 atom % stock material by 1 to 1 and 1 to 3 dilution and recrystallization, and burned and measured as described. The results from several runs are compiled in Table I. Actual isotope dilution experiments, in which the amount of nitrogen was determined in nicotinic acid, nicotinamide, and caffeine, are shown in Table II. Average size of unknown sample in these determinations was about 1.5 mg. The precision in the isotope dilution experiments is believed to be limited chiefly by the weighing error, which may be as high as ±2%, as samples and tracer as small as 1 mg. were weighed on a microbalance having a possible error of ±10 γ.

Table II. Per Cent Elemental Nitrogen Found by Actual Isotope Dilution Analysis

15 Atom % Tracer		30 Atom % Tracer	
Theor.	Found	Theor.	Found
	Nicotinic Acid		
11.38	11.11	11.38	11.10
	11.26		11.21
	11.31		11.72
	11.35		
	11.42		
	11.41		
	11.47		
Mean	11.33 ± 0.10		11.34 ± 0.22
Nicotinamide		Caffeine	
22.94	23.09	28.85	30.01
	23.00		28.98
	22.62		28.98
Mean	22.90 ± 0.22		29.32 ± 0.44

The background in the mass spectrometer itself may contribute an uncertainty of about ±0.5%, while residual nitrogen from the copper oxide and ampoule may contribute another ±0.5% error. These last two errors, however, will somewhat cancel out, as they affect the determination of the atom per cent excess nitrogen-15 in the tracer as well as in the unknown mixture. The contributions to the techniques of weighing ultramicro specimens made by the nuclear fission chemists and the current availability of practical commercial quartz fiber ultramicrobalances with sensitivities of a few micrograms would appear to solve these weighing difficulties.

By modifying the inlet system of the mass spectrometer to accommodate smaller gas samples it is believed that samples of

only a fraction of a milligram may be accurately analyzed by this technique.

By observing carbonization in the ampoule during combustion, and the background in the mass spectrometer in the mass range 27 to 52, it was found that 1 hour at 800° C. was a satisfactory time for the combustion procedure. A large peak at *m/e* 27 for some nitrogen compounds, as well as above average peaks in the mass range 47 to 52, was present when much shorter combustion times or lower temperatures were used.

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Isotope Dilution–Static Combination Method for Organic Carbon in Submilligram Specimens

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The method of elemental analysis described was undertaken to fill the need for a rapid, convenient, and precise means for quantitative determination of elemental carbon, especially where the available sample is very small, as in paper chromatography. The method enables one to determine elemental carbon in organic compounds with a precision comparable to that achieved in the standard micro-Pregl determination but with sample sizes of well under 1 mg. It is rapid, convenient, and easily adaptable to routine operation.

THE quantitative determination of the carbon content of submilligram specimens has become of ever-increasing importance because of the current availability and application of micromethods of isolation of new naturally occurring products.

Unterzaucher (*5*) described a method for the determination of carbon and hydrogen in submilligram quantities of material based on the determination of oxygen in the water and carbon dioxide resulting from the combustion of the sample. Kirsten (*4*) has stated, however, that Unterzaucher's procedure adds the troubles of the oxygen determination to those of the carbon and hydrogen determination.

Grosse, Hindin, and Kirshenbaum (*2*) introduced an isotope dilution procedure for the determination of carbon, oxygen, and nitrogen on 20- to 60-mg. quantities of organic compounds. The method provides for the static combustion of the sample after addition of a known quantity of $C^{13}O_2$, O_2^{18}, or N_2^{15} volumetrically, equilibration of the combustion gases, and subsequent measurement of the isotope ratio in the gas mixture.

Rather than measure the tracer volumetrically, it was felt that it would be more convenient to use the same method of measurement as that required for the sample. The method, herein presented, is based on the static combustion of an accurately weighed mixture of the unknown material and a known carbon-13–labeled tracer compound. The ratio of carbon dioxide of mass 45 to carbon dioxide of mass 44 is compared with the ratio for the gases obtained when the tracer alone is burned. The combustion is performed in evacuated, sealed Vycor ampoules in a furnace at 775° to 800° C. The ampoule, after completion of the combustion, is broken within the evacuated inlet system of the mass spectrometer and the $C^{13}O_2/C^{12}O_2$ ratio ascertained. Recently Wilzbach and Sykes (*6*) published a somewhat similar combustion technique for the ultimate determination of carbon-14.

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APPARATUS AND REAGENTS

The samples were weighed into platinum boats after it was ascertained that rather high carbon dioxide blanks were obtained when boats fashioned from electrolytic copper foil were employed.

The Vycor ampoules, ampoule breaker, and absorption tower were similar to those employed by Jones and Trenner (*3*), except that the absorption tower contained only Dehydrite.

A Consolidated-Nier mass spectrometer, Model 21-201, was used for the isotope ratio measurements after the gas-handling system (Figure 1) had been changed to include a Toepler pump, so that smaller volumes of gas could be manipulated and compressed to give a satisfactory inlet pressure (see Figure 1) and to allow for the removal of excess gas when necessary.

The cupric oxide was prepared by passing dry electrolytic oxygen over pure electrolytic copper powder at 800° C. in a silica tube.

Succinic acid, containing 30 atom % carbon-13, was prepared by refluxing overnight on the steam bath 3.5 ml. of ethylene bromide and 6.25 grams (20% excess) potassium cyanide (60 atom % C^{13}) in 40 ml. of 50% ethyl alcohol. Following distillation of the solvent and drying of the residue, the succinonitrile was extracted with four 50-ml. portions of absolute ethyl alcohol and filtered, and the solution was evaporated to dryness. The residue was treated with 50 ml. of concentrated hydrochloric acid and evaporated to dryness on the steam bath. The resulting succinic acid was extracted with four 50-ml. portions of methyl ethyl ketone. The solution was treated with activated Norite to decolorize it, filtered, and concentrated to crystallize the succinic acid. The acid was recrystallized from ethyl acetate, sublimed at 117° C. under vacuum, and recrystallized again from ethyl acetate. The filtered crystals were dried for 4 hours under vacuum (melting point = 189–192° C.). The yield was 57% (2.7 grams) based on the ethylene bromide.

ANALYTICAL PROCEDURE

The sample and a quantity of succinic acid tracer, containing approximately the same weight of carbon as that expected in the sample, are weighed accurately into a platinum boat. The boat and approximately 50 mg. of cupric oxide are put into a 6-inch Vycor ampoule marked with a diamond pencil for identification. After the lower half of the ampoule has been cooled in a dry ice–acetone bath, the center portion is heated in the oxygen-hydrogen flame to constrict it to capillary size for subsequent seal-off. The ampoule is connected to the manifold of a mercury diffusion pump and evacuated to a few microns before the final sealing at the constriction. Once sealed, the ampoule is rotated to effect an intimate mixture of the contents, and put into a furnace preheated to 775° to 800° C. for 1 hour.

After the completion of the combustion, the ampoule is removed from the hot furnace, cooled to room temperature, and transferred to the ampoule breaker, which is attached as shown in Figure 1 to the gas-handling train of the mass spectrometer.

After the entire inlet system has been evacuated for 5 minutes, with a mercury diffusion pump, all the stopcocks are closed, the ampoule is broken, the combustion gases are dried for approximately 1 minute over the Dehydrite in the absorption tower, and in the meantime the amplifiers of the instrument are balanced.

The dried combustion gases are then so compressed by means of the Toepler pump against the mass spectrometer that the ion voltage on collector 1 is equal to 20 volts. After the $C^{13}O_2$ of mass 45 is peaked on collector 2, at an ion-accelerating voltage of 1192 volts, the $C^{13}O_2/C^{12}O_2$ or mass 45/44 ratio is read directly on the instrument panel. Several ratio values are obtained and the average value is used in the calculations.

After the average ratio value has been multiplied by the f factor of the mass spectrometer (*1*) and corrected for the natural abundance of oxygen-17 and oxygen-18, it is converted to atom per cent excess carbon-13 in the usual manner.

The mass spectrometer measures:

$$\frac{\text{Mass 45}}{\text{Mass 44}} = \frac{C^{13}O_2}{C^{12}O_2} = r = \frac{C^{13}}{C^{12}} \tag{1}$$

If f is the fraction of atoms of a given kind, then

$$f_{C^{13}} = \frac{C^{13}}{C^{13} + C^{12}} = \frac{r}{r+1} \tag{2}$$

$$f_{C^{13}} = 1 - f_{C^{13}} = \frac{1}{r+1} \tag{3}$$

A study of the carbon-13 content of the succinic acid tracer, carried out using varied specimen weights, revealed that there was a small but significant carbon contamination (background) involved in the apparatus and technique. This, of course, became of greater importance as the tracer specimen weight used in a given combustion was decreased. This background effect was evaluated in the following manner:

Let T refer to the tracer, whence the number of mole atoms of carbon-13 is given by:

$$C^{13} = \frac{W_T}{M_T} \times n_C^T \times \frac{r_T}{r_T + 1} + \frac{W_C \times 1.11 \times 10^{-2}}{12.01} \tag{4}$$

and for carbon-12 is:

$$C^{12} = \frac{W_T}{M_T} \times n_C^T \times \frac{1}{r_T + 1} + \frac{W_C \times 0.989}{12.01} \tag{5}$$

where

W_T = weight (in mg.) of tracer in a given combustion
M_T = molecular weight of the succinic acid tracer
n_C^T = number of carbon atoms per molecule of tracer
r_T = true C^{13}/C^{12} ratio of the tracer at large specimen weights, where the background is negligible
1.11×10^{-2} and 0.989 = known natural abundances of C^{13} and C^{12}, respectively
W_C = weight (in mg.) of the carbon background

Let $r_T^1 = \frac{C^{13}}{C^{12}}$ be the mass spectroscopically observed ratio for a given combustion of this tracer only. Then by Equations 4 and 5

$$W_C = \frac{12.01\, W_T\, n_C^T\, (r_T - r_T^1)}{M_T(r_T + 1)\,(0.989\, r_T^1 - 1.11 \times 10^{-2})} \tag{6}$$

and

$$M_T = 13\, n_C^T \times \frac{r_T}{r_T + 1} + 12\, n_C^T \times \frac{1}{r_T + 1} + 1.008\, n_H^T + 16\, n_O^T$$

For the succinic acid tracer of $C_4H_6O_4$

$$r_T = 0.4576;\ n_C^T = 4;\ n_H^T = 6;\ n_O^T = 4$$

whence $M_T = 119.3$

Equation 6 then reduces to:

$$W_C = \frac{0.2761\, W_T(0.4576 - r_T^1)}{0.989\, r_T^1 - 1.11 \times 10^{-2}} \tag{7}$$

Table I shows the application of Equation 7 to the experimental observations carried out on this tracer. The results clearly

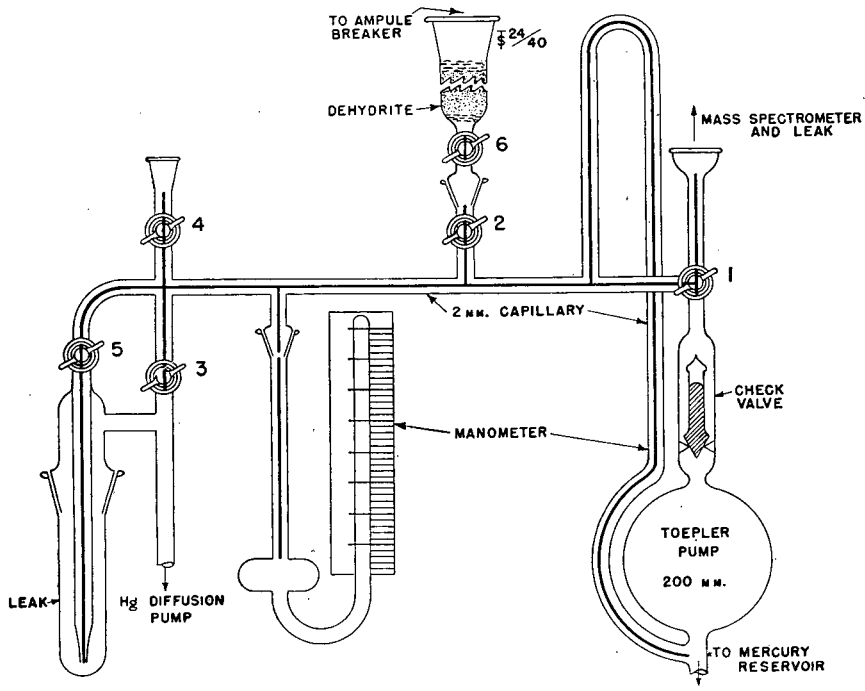

Figure 1. Modified gas-handling system for Consolidated-Nier mass spectrometer

illustrate the statistical constancy and magnitude of the background effect encountered in this work.

In view of the good constancy of W_C it is appropriate to derive the following corrected equation for use in computing carbon analyses:

$$C^{13} = \frac{W_T}{M_T} \times n_C^T \times \frac{r_T}{r_T + 1} + \frac{W_S}{M_S} \times n_C^S \times 1.11 \times 10^{-2} + \frac{W_C}{12.01} \times 1.11 \times 10^{-2} \quad (8)$$

$$C^{12} = \frac{W_T}{M_T} \times n_C^T \times \frac{1}{r_T + 1} + \frac{W_S}{M_S} \times n_C^S \times 0.989 + \frac{W_C}{12.01} \times 0.989 \quad (9)$$

$$\frac{C^{13}}{C^{12}} = r_S \quad (10)$$

where W_S, M_S, and n_C^S are the same quantities as used above but refer to the sample being analyzed. r_S is the observed mass spectroscopic ratio in the combustion of a sample plus tracer mixture. If X_C is the quantity sought—i.e., the weight fraction of carbon in the sample—then

$$X_C = \frac{12.01\, n_C^S}{M_S} \text{ or } \frac{n_C^S}{M_S} = \frac{X_C}{12.01} \quad (11)$$

Combining Equations 8, 9, 10, and 11 and solving for X_C one gets:

$$X_C = \frac{12.01}{W_S(0.989\, r_S - 1.11 \times 10^{-2})} \times \left[\frac{W_T n_C^T (r_T - r_S)}{M_T[r_T + 1)} + \frac{1.11 \times 10^{-2} W_C}{12.01} \times \frac{-0.989 W_C r_S}{12.01}\right] \quad (12)$$

Table I. Carbon Background Effect

W_T	$r_{\frac{1}{2}}$ (Obsd.)	$W_C \times 10^3$
0.187	0.4444	1.64
0.216	0.4434	2.04
0.233	0.4456	1.86
0.363	0.4473	2.49
0.380	0.4507	1.76
0.423	0.4503	2.07
	Mean	1.98 ± 0.22

As in this work $r_T = 0.4576$, $n_C^T = 4$, $M_T = 119.3$, and $W_C = 1.98 \times 10^{-3}$, Equation 12 becomes:

$$X_C = \frac{1.98 \times 10^{-3}}{(89.1 r_S - 1)W_S} \times [1.257 \times 10^4\, W_T\, (0.4576 - r_S) - 89.1 r_S + 1] \quad (13)$$

the "corrected" equation used for computing the analytical results given in Table II, which shows this method applied to compounds of known carbon content and varied structural types.

In carrying out isotope dilution assays of this kind maximum mass spectroscopic precision is achieved (*2*) when

$$r_S \cong 1/2\, r_T$$

Thus for an average organic compound where, generally, $X_C = 0.50$, Equation 13 dictates that the quantity $\frac{W_T}{W_S}$ should be kept at about 1.7.

Table II. Determination of Carbon in Known Compounds

Compound	Carbon, %	Sample Wt., Mg.	Tracer Wt., Mg.	r_S	Carbon Found, %	Mean
Succinic acid	40.7	1.307	0.940	0.1572	41.2	
		1.228	1.047	0.1752	40.9	
		0.910	0.986	0.1998	40.4	
		0.878	0.932	0.1990	40.6	
		0.719	0.684	0.1880	40.3	
		0.571	0.540	0.1861	40.7	
		0.497	0.902	0.2587	40.4	
		0.447	1.521	0.2101	40.1	
		0.255	0.260	0.1938	40.4	
		0.156	0.181	0.2059	40.6	40.6 ± 0.3
Benzamide	69.4	0.987	1.356	0.1682	70.4	
		0.913	1.390	0.1797	69.9	70.1 ± 0.2
P-Nitrobenzoic acid	50.3	1.177	1.958	0.2251	50.3	
		0.881	1.151	0.1965	51.1	
		0.804	1.453	0.2335	50.7	
		0.167	0.144	0.1555	49.2	50.3 ± 0.3
Nicotinic acid	58.5	1.186	1.232	0.1602	57.8	
		0.781	0.990	0.1786	58.8	
		0.661	1.323	0.2290	58.4	58.3 ± 0.2
Cystine	30.0	1.254	1.192	0.2192	30.3	
		1.059	1.694	0.2788	29.7	
		0.481	0.462	0.2218	29.7	
		0.395	0.565	0.2657	29.6	
		0.385	0.345	0.2135	29.7	
		0.365	0.204	0.1594	30.8	30.0 ± 0.2
Cortisone	69.9	0.542	0.862	0.1849	69.4	
		0.509	0.818	0.1852	69.9	69.6 ± 0.2
9α-Fluorohydrocortisone acetate	65.4	0.731	1.042	0.1783	66.3	
		0.560	0.588	0.1483	65.8	66.0 ± 0.2

SUMMARY

The proposed method has made possible the quantitative determination of carbon in organic compounds on samples as small as 200 γ with an accuracy within 1% using an Ainsworth microanalytical balance. Recently a Model E ultramicrobalance has been obtained which is sensitive to 0.05 γ. This quartz fiber torsion balance is supplied by Microtech Services Co, Box 121, Berkeley, Calif., and is excellent for this type of analysis.

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Displacement of the Nitro Group during Determination of Nitrophenols and Nitroanalines by the Koppeschaar Method—Correction

In the article on "Displacement of the Nitro Group during Determination of Nitrophenols and Nitroanilines by the Koppeschaar Method [ANAL. CHEM. **27**, 1494 (1955)], the eighth equation on page 1494 should read:

$$2NO + O_2 \rightarrow 2NO_2$$

The equation on page 1498 should read:

$$HgO + 2Br_2 + H_2O \rightarrow 2HOBr + HgBr_2$$

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Radiometric Determination of Inorganic Fluoride

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Sodium fluoride and sodium hexafluosilicate are titrated with samarium ion containing europium carrier-tracer. The end point is determined by measuring the excess of titrant by a radiometric procedure. Under optimum conditions, 20 to 30 mg. of fluoride can be determined with an error of less than 1%. Dilution of the fluoride by a factor of 5 results in an error generally less than 3%. Errors in the determination of sodium hexafluosilicate are greater than with sodium fluoride. Colloid flocculation is usually incomplete. The colloid remaining in suspension leads to low results. Acetic acid helps considerably in flocculating the colloid, but many other flocculents cause coprecipitation and high results. The titration can also be done on a micro scale. Fluoride in the amount of 38 γ is determined with generally less than 2% error.

THE fluoride method of Willard and Winter (*7*) has been used with a great deal of success. Recently Popov and Knudson (*5*) reported a gravimetric method for fluoride in which lanthanum ion was used as the precipitant. A radiometric-volumetric method is presented here which employs samarium ion as the precipitant and europium carrier-tracer to detect the end point.

A γ-emitting tracer is ideal for tracer work, because solutions can be handled and counted directly in test tubes. High counting efficiency (of the order of 5%) with a modern scintillation anticoincidence counter is attained. Counting errors can be reduced to less than 1%.

A radiometric procedure (*3*) offers some advantage over other instrumental procedures. The specific activity of the tracer can be changed between extreme limits, so that microdeterminations can be carried out with accuracy determined primarily by the microanalytical equipment and the chemistry of the titration. Amperometric and conductometric titrations and similar methods which depend on properties of an ion or ions in solution are disadvantageous because the property being measured to detect the end point usually cannot be varied between wide limits. These methods require auxiliary equipment in the solution; hence, there is a physical limitation to the amount of solution employed. Smaller volumes could probably be used with a radiometric procedure.

The radiometric procedure also has disadvantages. It is limited to methods where there is an actual physical separation of the unknown from the solution phase, such as a precipitation reaction. For counting, the solution phase must be well shielded from the separated phase, as has been done by Langer (*2*), or an aliquot of the solution may be removed and counted apart from the vicinity of the titration (*3*).

The counting of an aliquot removed from solution was the procedure used in this work. Every point on the titration curve, however, requires a separate sample, unless the aliquot is returned each time. Hence, for a good end point, three or four times as much unknown is required as with other procedures. In a routine titration, two points probably would suffice to determine the end point (*2*).

Lanthanum forms insoluble compounds of definite stoichiometric composition (*1, 5*) with fluoride ion. Some of the lanthanons also have isotopes which have excellent characteristics as tracers. Of the lanthanons which are high energy γ-emitters, europium has a suitable half life of 12.4 years (*4*). However, this lanthanon is so expensive that it was decided that the titration would be more useful if a much cheaper juxtalanthanon, samarium, was used as the main precipitant, with europium as the carrier-tracer.

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EXPERIMENTAL

Chemicals. Sodium fluoride, Baker and Adamson, was used for most of the determinations. It was heated at 500° to 600° C. for several hours immediately before weighing. Pure sodium fluoride was prepared by the procedure given by Reynolds and Hill (*6*), and was used for most of the microtitrations.

Sodium hexafluosilicate, Baker's analyzed, was dried at 110° C. for about 15 hours prior to use.

Samarium oxide, containing 1.5% europium oxide, was obtained from the Société de Produits Chimique des Terres Rares, Paris, France. Stock solutions were made by dissolving the freshly ignited oxide (ignited at 950° C. for about 5 hours) in dilute nitric acid and adding the tracer and acetic acid as required. The amount of europium added in the tracer was insignificant.

Tracer. Pure europium oxide from Johnson, Matthey, and Co., New York, was subjected to neutron irradiation in the Los Alamos water boiler. The specific activity was about 25 mc. per gram.

Equipment. An International clinical centrifuge was used. For microwork, a Misco air-driven centrifuge (Microchemical Specialties Co., Berkeley, Calif.) was used. Centrifuge tubes of about 0.5-ml. capacity were made of capillary tubing.

Counting was done with a scintillation anticoincidence counter made by Group CMR-7 of this laboratory. The samples were surrounded by about 4 inches of lead during counting. Time of

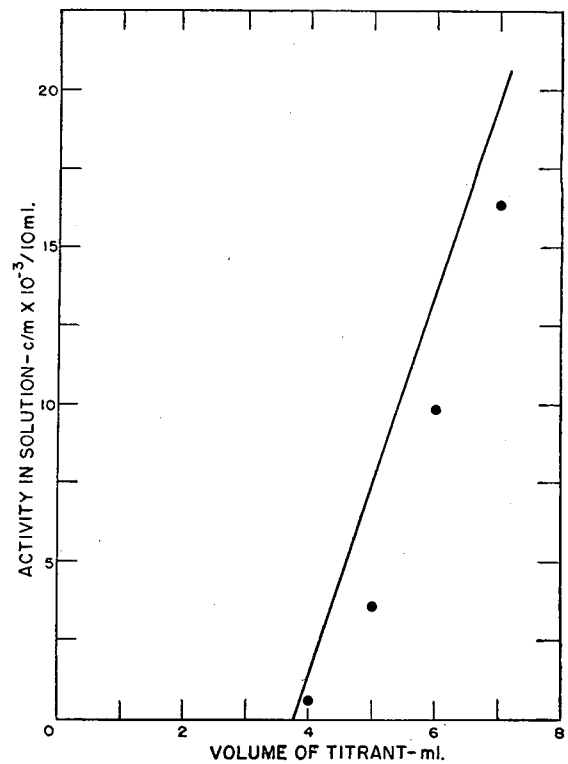

Figure 1. Titration of 20 ml. of 0.04296*M* sodium fluoride containing 0.05*M* citric acid with 0.07528*M* rare earth nitrate in 1*M* acetic acid

Line represents ideal extrapolation

Table I. Determination of Sodium Fluoride

(Titrant, 0.1004*M* rare earth nitrate, pH about 2, samples not heated before centrifugation)

Centrifuge, Time, Hr.	Fluoride Molarity	Taken, mg.	Found, mg.	Error, %
2	0.093	28.3	28.1	−0.6
2	0.093	28.3	26.7	−5.7
3½	0.093	28.3	26.6	−6.0
3	0.093	28.3	27.5	−3.0

counting was 5 or 10 minutes and the total count was sufficiently high to reduce errors to less than 1%.

A Gilmont microburet was used for delivery of the titrant in the microtitrations. The 0.1-ml. delivery tube was replaced with one of a larger diameter so that the buret could be more easily filled. This tube was made in the laboratory glass shop.

Necessity for the control of pH in fluoride determinations has been stressed by Popov and Knudson (*5*). In this work the pH was kept between 1.8 and 3.5. Acetic acid was used to control the pH and also to serve as a colloid flocculent. Measurements of pH were made with a Model G Beckman instrument.

RESULTS AND DISCUSSION

Titration of Sodium Fluoride. Although the microdetermination was done first, the results on the conventional titration are presented first to show the difficulties likely to be encountered. Popov and Knudson (*5*) found that colloidal lanthanum fluoride is usually formed when precipitating fluoride ion with lanthanum ion. They brought down the colloid by using an acetic acid flocculent and by heating the solutions. The same difficulty was encountered in this work.

Fifteen milliliters of neutral sodium fluoride solution was added to each of four 40-ml. centrifuge tubes, followed by 10 ml. of 1*M* acetic acid. Increasing amounts of titrant were added to the fluoride aliquots, so that the end point was exceeded in each case. Each solution was stirred with a stirring rod, which was removed without washing. The samples were placed in the centrifuge and revolved at about 3000 r.p.m. to separate the fluoride precipitate. A 10-ml. aliquot of the supernatant liquid was removed, placed in a counting test tube, and counted. The activity was corrected for dilution by the titrant. A plot was made of activity in the supernatant solution *vs.* volume of titrant, and the end point was determined from the extrapolated line.

Table I shows the results of some titrations which were done without heating. The results are all low, showing that extended centrifugation does not remove all of the precipitated rare earth fluoride.

Other flocculents were tried in an effort to effect coagulation and separation of the precipitate rapidly at room temperature. The colloid should have a positive charge on the excess rare earth ion side of the end point; hence, the anions of citric acid, *l*-malic acid, and *d*-tartaric acid were tried. Citric acid flocculated the precipitate nicely but also caused coprecipitation of rare earth citrate, as shown in Figure 1. The line represents the ideal extrapolation as calculated from the tracer activity in the titrant. The points are all low past the end point, and reflect the decrease in activity in the solution caused by the removal of rare earth ion by coprecipitation.

Because malic acid complexed the rare earth ion and prevented precipitation with some samples, it was ineffective as a flocculent. Tartaric acid did not completely flocculate the colloid.

Work on finding a flocculent was discontinued because of the coprecipitation problem. However, for a practical method, the coprecipitation may not be objectionable. Figure 1 shows that an extrapolation in the immediate vicinity of the end point (which is within 0.5 ml.) should give a result close to the stoichiometric end point. On the other hand, an extrapolation from the data taken far from the end point gives a result which is much in error.

Heating of the samples prior to centrifugation was tried as a method of flocculating the colloid. The procedure was similar to that used for samples without heating. A 20-ml. sample was taken, except for sodium fluoride which was 0.0993*M*, when a 15-ml. sample was taken. Titrant was added to each of the four samples to exceed the end point, and the samples were stirred. With the stirring rod still in each sample, they were heated in a water bath for 15 minutes at 60° to 80° C., stirred again, and allowed to cool to room temperature. The samples were centrifuged and aliquots taken as before.

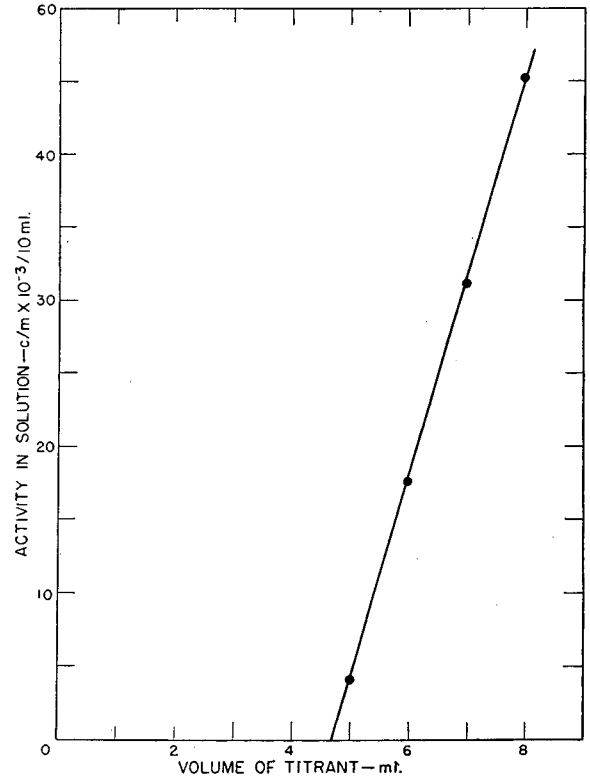

Figure 2. Titration of 15 ml. of 0.0993*M* sodium fluoride with 0.1061*M* rare earth nitrate in 1*M* acetic acid

Line represents ideal extrapolation

Table II shows that reasonable results were obtained with the more concentrated fluoride solutions. Errors in the determinations were less than 1%, and deviations from the ideal titration plot were minor, as can be seen by comparing the line in Figure 2 to the data.

With 0.01*M* sodium fluoride, a greater error was encountered in the determinations, even after heating the samples according to the method just described. Table III gives the results, and Figure 3 shows in detail what causes low results. There is considerable deviation from ideality on the upper portion of the plot.

Table II. Determination of Sodium Fluoride

(Titrant, 0.1061*M* rare earth nitrate in 1*M* acetic acid, pH 2.33, samples heated before centrifugation)

pH of Counting Sample[a]	Fluoride Molarity	Taken, mg.	Found, mg.	Error, %
2.8	0.0555	21.09	21.18	+0.5
2.8	0.0555	21.09	21.23	+0.7
2.9	0.0572	21.74	21.68	−0.3
2.5	0.0993	28.30	28.42[b]	+0.4

[a] Value for sample containing largest amount of titrant. Sample containing least amount of titrant would have pH as much as 0.2 unit higher.
[b] Plotted in Figure 2.

Table III. Determination of Sodium Fluoride

(Titrant, 0.0212*M* rare earth nitrate in 1*M* acetic acid, pH 2.35, samples heated before centrifugation)

pH of Counting Sample[a]	Fluoride Molarity	Fluoride Taken, mg.	Fluoride Found, mg.	Error, %
3.1	0.0111	4.22	4.26	+1.0
3.1	0.0111	4.22	4.11	−2.3
3.0	0.0111	4.22	4.14	−2.0
3.0	0.0111	4.22	4.14	−2.0
3.1	0.01108	4.21	4.26	+1.2
3.0	0.01108	4.21	4.11	−2.3
3.0	0.01108	4.21	4.13	−2.0
3.0	0.01108	4.21	4.11	−2.3
2.9	0.01108	4.21	4.12[b]	−2.1
3.3[c]	0.0111	4.22	4.03	−4.6
3.4[c]	0.0111	4.22	4.12	−2.4
3.4[c]	0.0111	4.22	4.23	+0.3
3.4[c]	0.0111	4.22	4.23	+0.3
			Av.	1.9

[a] Value for sample containing the largest amount of titrant. Sample containing least amount of titrant would have pH as much as 0.2 unit higher.
[b] Plotted in Figure 3
[c] pH of titrant, 2.72.

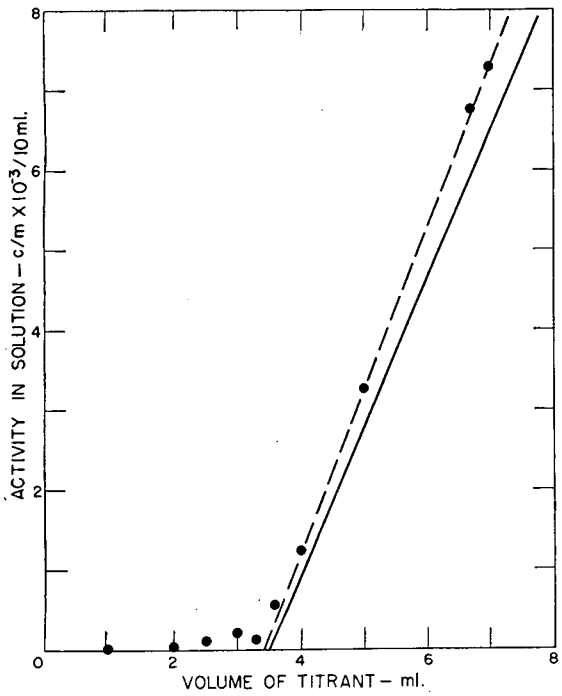

Figure 3. Titration of 20 ml. of 0.01108*M* sodium fluoride with 0.0212*M* rare earth nitrate containing 1*M* acetic acid

Solid line represents ideal extrapolation; dashed line gives experimental end point

However, the data follow a straight line which has a different slope from the ideal line. The amount of colloidal fluoride not separated from the solution appears to be directly proportional to the amount of excess rare earth ion present. Thus, at the end point (the isoelectric point of the colloid) the experimental line extrapolates close to the ideal line and the error is not large, though appreciable. The hump in the plot of the data has significance and is approximately reproducible. Here a negatively charged colloid is formed, which also diminishes in quantity as the isoelectric point is approached.

The amount of colloid left in solution as shown by Figure 3 is not detected when the amount of fluoride is 5 to 10 times greater. This is perhaps the reason that more concentrated fluoride gives better results. More dilute solutions were not investigated because of anticipated difficulties of colloid separation, which were actually shown experimentally in the microtitrations.

The limiting factor in this particular method appears to be the colloid flocculation problem, rather than the solubility limitation which is present in other similar titrations.

Determination of Sodium Hexafluosilicate. The method developed for sodium fluoride was tried on sodium hexafluosilicate in order to find if the titration could be performed on the distillate of a Willard-Winter (7) separation. Results of these titrations are given in Table IV; the procedure used was the same as that used previously for samples which were heated before centrifuging. All of the results are low, showing that separation of the colloid is incomplete.

Table IV. Determination of Sodium Hexafluosilicate

(Samples heated)

pH of Counting Sample[a]	Hexafluosilicate Molarity	Hexafluosilicate Taken, mg.	Hexafluosilicate Found mg.	Error, %
Titrant, 0.2248*M* rare earth nitrate in 1*M* acetic acid, pH 1.92				
2.1	0.01669	62.80	57.5	− 8.4
Titrant, 0.0212*M* rare earth nitrate in 1*M* acetic acid, pH 2.32				
2.7	0.00213	8.00	7.60	− 5.0
2.7	0.00213	8.00	7.56	− 5.5
2.7	0.00213	8.00	7.74	− 3.2
2.6	0.00213	8.00	7.18	−10.2

[a] Value for sample containing largest amount of titrant. Sample containing least amount of titrant would have pH as much as 0.2 unit higher.

Acetic acid was added to the samples in an effort to facilitate flocculation of the colloid. Results of these tests are given in Table V. The same procedure was used as for other heated samples, except that the volume of sample was different, and acetic acid was added to the sodium hexafluosilicate.

Acetic acid helps in the flocculation, but deviations from ideality are greater than with sodium fluoride. Figure 4 shows the effect of the acid in flocculating the colloid, especially near the end point. With the larger amount of acetic acid present, the amount of unseparated colloid is at a minimum near the end point, but increases on either side. The shape of the plot (square

Table V. Determination of Sodium Hexafluosilicate

(Titrant, 0.1124*M* rare earth nitrate in 0.5*M* acetic acid, pH 2.28, acetic acid added for flocculation)

pH of Counting Sample[a]	Molarity of Acetic Acid in Hexafluosilicate	Hexafluosilicate Molarity	Hexafluosilicate Taken, mg.	Hexafluosilicate Found, mg.	Error, %
2.4	0	0.008345	31.40	30.0	−4.4
2.0	0.8	0.01391	31.40	30.3	−3.4[b]
2.1	1.3	0.01113	31.40	31.1	−1.1
2.1	1.3	0.01113	31.40	30.7	−2.4[c]
2.1	2.0	0.008345	31.40	30.7	−2.4

[a] Value for sample containing largest amount of titrant. Sample containing least amount of titrant would have pH as much as 0.2 unit higher.
[b] Plotted as circles in Figure 4.
[c] Plotted as squares in Figure 4.

Table VI. Microdetermination of Sodium Fluoride

(Titrant, rare earth nitrate in excess nitric acid to give pH approximately 0.3)

Rare Earth Nitrate, Molarity	Fluoride Molarity	Fluoride Taken, γ	Fluoride Found, γ	Error, %
0.500	0.1000	376	374	−0.5
0.500	0.1000	376	376	0
0.500	0.1000	376	367	−2.3
0.500	0.1000	376	379	+0.8
0.500	0.1000	376	382	+1.5
0.250	0.0500	285[a]	288	+1.0
0.250	0.0500	190	190	0
0.0202	0.0100	38.0	38.5	+1.2
0.0202	0.0100	38.0	37.8	−0.5
0.0202	0.0100	38.0	38.6	+1.6[b]
			Av.	0.94

[a] 300 μl. of fluoride sample taken.
[b] Plotted in Figure 5.

symbols) in Figure 4 is similar to that in Figure 3 for sodium fluoride.

It appears that silica formed when the fluoride is precipitated by rare earth ion tends to stabilize the colloid. Acetic acid helps in reducing the stability of the colloid, but not sufficiently to allow an accurate determination of the end point.

Microdetermination of Sodium Fluoride. Some difficulty was encountered in the initial work in flocculating the colloid. Acetic acid, recommended by Popov and Knudson (*5*), proved to be the best flocculent tried, although moderate concentrations of dichromate also helped. Pyrophosphate, ferro-, and ferricyanide, when tried as flocculents, coprecipitated as the rare earth salt on the excess rare earth side of the end point.

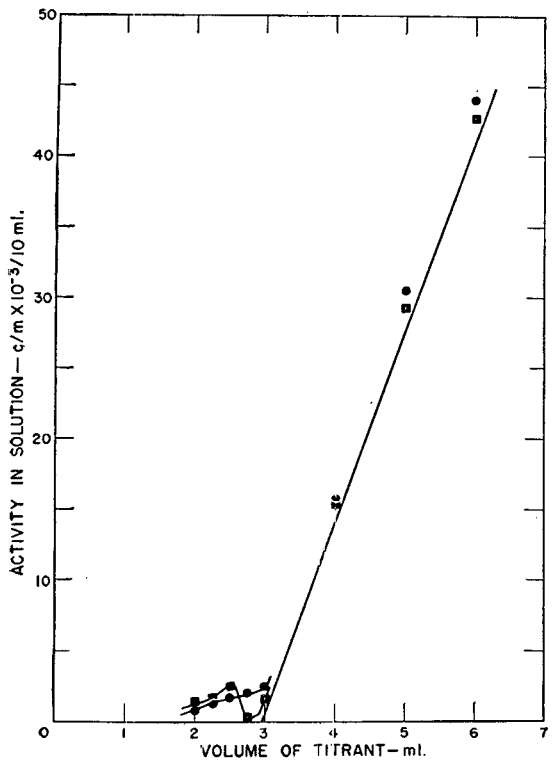

Figure 4. Effect of acetic acid in the determination of sodium hexafluosilicate

Circles represent titration of 12 ml. of 0.01391*M* sodium hexafluosilicate containing 0.8*M* acetic acid with 0.1124*M* rare earth nitrate containing 0.5*M* acetic acid. Squares represent titration of 15 ml. of 0.01113*M* sodium hexafluosilicate containing 1.3*M* acetic acid with same titrant. Line represents ideal extrapolation

In Table VI are data obtained on several titrations which were done according to the following procedure:

Titrant was added in increasing amounts with a Gilmont microburet to a separate centrifuge tube, followed by neutral sodium fluoride from a calibrated 200-μl. pipet. Flocculent, consisting of approximately 0.024*M* sodium acetate in 1*M* acetic acid, was added in the amount of 100 or 105 μl. The samples were stirred with a platinum wire, and centrifuged for 30 to 40 minutes at about 20,000 r.p.m. A 200-μl. aliquot of the supernatant liquid from each sample was added to a counting tube, diluted to 10 ml., then counted 5 or 10 minutes. The activity was corrected for dilution by the titrant, then a plot was made of the activity in the supernatant solution *vs.* volume of titrant added. The end point was determined from the extrapolated line.

It may be noted here that the flocculent could have been added as a solution in the titrant rather than separately. Also, the order of adding the reagents should not influence the end point. Heating of the samples was not necessary, because the centrifuge removed most of the precipitate. By comparing Figure 5 to Figure 3, it is seen that more of the colloid is removed by the smaller centrifuge. The line in Figure 5 is drawn through the data to give the end point rather than to depict the ideal titration line. Some deviation from the ideal line might be expected at points relatively far removed from the end point.

Table VII. Microdetermination of Sodium Fluoride

(Effect of cation impurities)

Impurity	Mole Impurity per Mole Fluoride	Fluoride Taken, γ	Fluoride Found, γ	Error, %
Fe^{+++}	0.2	190	172	− 10
Fe^{+++}	0.2	190	168	− 11
Ni^{++}	0.2	190	≈200	+≈5
Cr^{+++}	0.23	190	94	− 50
Cr^{+++}	0.23	190	94	− 50

Extending the method to include the titration of 0.001*M* fluoride was not successful. In one titration in which 0.001*M* fluoride was titrated with 0.0025*M* pure europium, the plot went through the origin, showing that no europium fluoride was separated from solution. In a duplicate experiment, a rough end point 63% low was obtained.

From the expected solubility of europium fluoride [obtained by comparison with gadolinium fluoride (*1*)], it was predicted that 0.003*M* fluoride could be determined with less than 2% error. From the plot in Figure 5, it could be expected that a fairly accurate determination could be made even with tenfold dilution. However, the limiting factor appears to be colloid flocculation and separation, not the solubility.

Effect of Impurities on Microtitration. The effect of some common cation impurities was investigated in conjunction with the microprocedure. In Table VII are results obtained with iron, nickel, and chromium. Iron interfered by complexing part of the fluoride and giving a low result. The data for the titra-

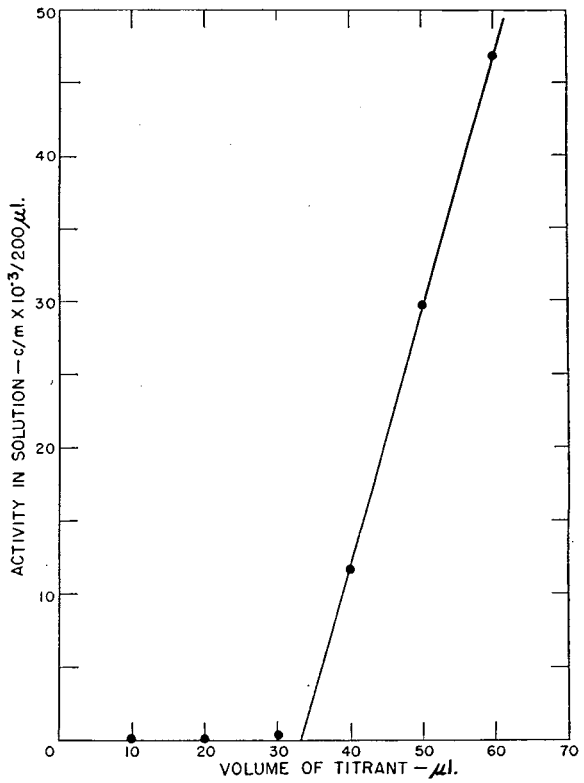

Figure 5. Microtitration of 200 μl. of 0.01*M* sodium fluoride with 0.0202*M* rare earth nitrate

Line is actual extrapolation, not ideal line

tions followed a straight line which was parallel to the line expected if no iron were present. Hence, the amount of fluoride complexed by iron remained constant and independent of the amount of rare earth ion present.

Nickel also interfered, but not in a predictable fashion. The data on nickel are given to show that it interferes, but they are not necessarily a guide for estimating its effect. The data for the titration deviate from a straight line and give a poor end point. Therefore, nickel should be eliminated prior to the determination of fluoride.

Chromium appears to interfere in a predictable fashion. Data in Table VII show that about half of the fluoride is removed as a nontitratable species. The data show that for each mole of chromium present, there are about 2 moles of fluoride removed as a complex ion; hence, the interfering species can be depicted as difluochromium(III) ion, which Wilson and Taube have described (*8*). According to their data, this ion should be formed in high yield under the conditions of the experiment described here. As with iron, the titration lines were parallel to the line expected if no chromium were present.

Improvement of Procedure. It should be possible to improve the accuracy of the titrations by measuring the excess titrant much closer to the end point—e.g., within 10 or 20% excess of titrant.

As lanthanum fluoride is more insoluble than samarium fluoride (*1*), perhaps more dilute fluoride could be titrated with lanthanum ion. In order to ensure complete carriage of the europium tracer, the precipitation and flocculation should be rapid. Radioactive lanthanum-140 could be used as the tracer, but its half life of 40 hours is too short to be practical for routine work.

ACKNOWLEDGMENT

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Spot Test for Diketones and Quinones Based on Catalytic Effect

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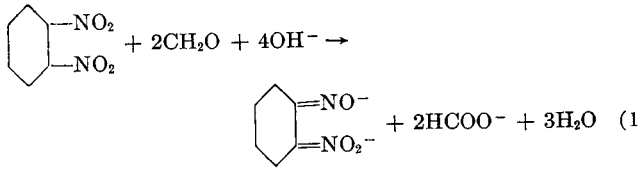
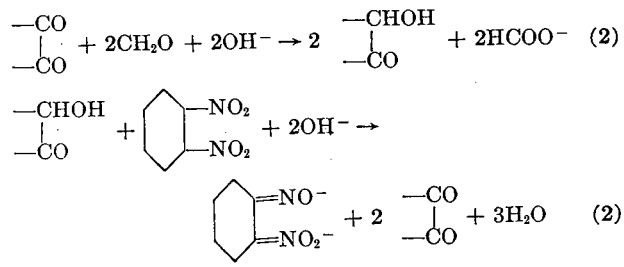
The slow reaction between formaldehyde and 1,2-dinitrobenzene, which yields a violet alkali salt of the aci- form of *o*-nitrosonitrobenzene, is hastened by the addition of 1,2-diketones and quinones. It is assumed that an intermediate catalysis is involved. Microgram quantities of the catalytically active compounds can be detected by drop reactions if certain simple conditions are maintained. New microtests for anthracene, phenanthrene, and inositol are made possible by the ready conversion of these compounds into anthraquinone, phenanthraquinone, and cyclic polyketones, respectively.

FORMALDEHYDE functions as a hydrogen donor in alkaline solution and thus reduces 1,2-dinitrobenzene to the aci- form of 1,2-nitrosonitrobenzene (*1*), giving a violet alkali salt. However, the reaction



proceeds slowly even with relatively large quantities of formaldehyde in sodium carbonate solution, and hence, has no analytical value. The redox reaction has been found to be rapid in the presence of 1,2-diketones and quinones, which act as catalysts. This effect is the basis of a sensitive and specific test, which may be used as a spot reaction, for these compounds.

The catalytic hastening of Equation 1 by 1,2-diketones may be due to the fact that formaldehyde in alkaline solution reduces them to 1,2-hydroxyketones as shown in Equation 2, and these products in turn reduce the 1,2-dinitrobenzene to the violet *o*-quinoidal alkali salt as shown in Equation 3. The diketone is thus regenerated and can react again according to Equation 2. Reactions 2 and 3, which occur again and again, proceed faster than Reaction 1. Addition of the partial Reactions 2 and 3 gives a net reaction which is identical with Reaction 1, in which the diketone does not appear, even though it is continuously consumed and regenerated.



There is no doubt as to the existence of Reaction 3, because microgram quantities of acyloins and benzoins (compounds containing the group —CHOH—CO—) have been found to yield the violet color characteristic of organic hydrogen donors when they are warmed with an alkaline-alcohol solution of 1,2-dinitrobenzene (*2*). No reports could be found in the literature about the reduction of diketones by formaldehyde as in Reaction 2. Attempts to convert benzil quantitatively into benzoin by warming an alcohol solution with a sodium carbonate solution of formaldehyde failed. However, the evaporation residue of such a reaction mixture contained benzoin, as revealed by the color reaction with 1,2-dinitrobenzene and also by the production of hydrogen

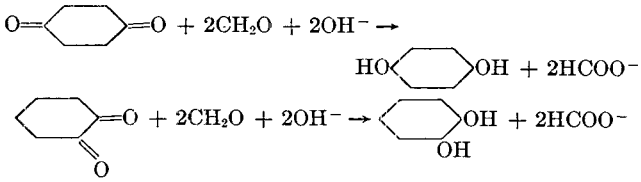
sulfide on heating with sulfur to 140° C. Production of hydrogen sulfide on fusing with sulfur is characteristic of benzoin and for compounds with secondary alcohol groups (*3*).

A comparison test with the evaporation residue of a sodium carbonate–formaldehyde solution gave no color reaction with 1,2-dinitrobenzene and only a weak hydrogen sulfide reaction after heating with free sulfur. The latter response was due to the formation of some hydrogen sulfide when hydrous sodium carbonate plus sulfur is heated to 140° C., by the action of the superheated water vapor released at this temperature. These findings are additional supporting evidence for the mechanism proposed above. Despite the fact that Reaction 2 gives but slight yields of 1,2-hydroxyketone, its participation in the catalysis is entirely feasible. The decisive factor is not extensive reaction, but only whether such amounts of 1,2-hydroxyketones as are produced can participate rapidly in Reaction 3.

DETECTION OF 1,2-DIKETONES AND QUINONES

If the catalytic action of 1,2-diketones is to be employed in analysis, it is necessary to maintain conditions at which the uncatalyzed redox reaction, Equation 1, proceeds with the lowest possible velocity, because the test is based on the establishment of the differences in the reaction rates. After many trials it was found that the greatest reliability and sensitivity are attained by conducting the reaction in strong carbonate solution at the temperature of a boiling water bath, and by using a benzene solution of 1,2-dinitrobenzene. This may be obtained in surface-rich form by evaporating off the benzene and dissolving the residue in hot water to form an approximately 0.4*N* solution. Under these conditions, Reaction 1 becomes apparent only after 4 to 5 minutes, whereas even minimum amounts of catalytically active diketones produce the color reaction much sooner. Solutions of the test material may be used if necessary.

It was found that quinones exhibit a similar catalytic action. This is probably ascribable to the fact that these quinones lead to hydroxy compounds via redox reactions which are analogous to Reaction 2.



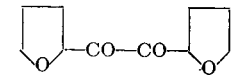
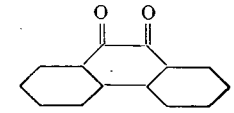
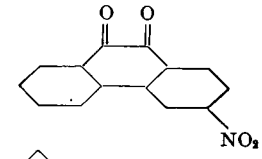
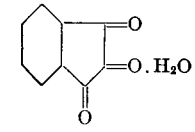
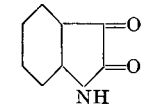
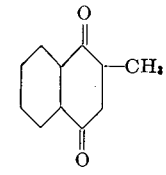
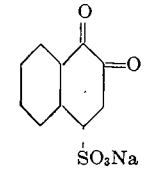
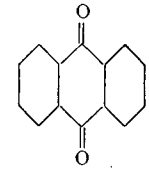
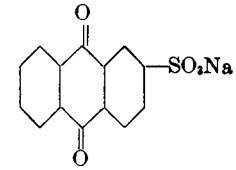
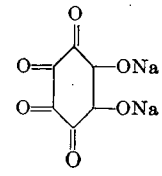
These products function as hydrogen donors to 1,2-dinitrobenzene and so bring about the color reaction. Consequently, it is advisable to make a preliminary trial with the sample in the absence of formaldehyde, using the prescribed procedure. If no color appears, or a pale violet at most, the test should be repeated with the inclusion of formaldehyde. A violet color or a more intense result of the color reaction is then proof of the presence of catalytically active 1,2-diketones or quinones.

A simple procedure for detecting 1,2-diketones in the presence of numerous reducing agents, which give the color reaction without the addition of formaldehyde, consists of adding alkali hypobromite to oxidize the sample and then proceeding with the catalysis reaction. (Compare with the detection of inositol in the presence of reducing sugars, described later.) However, the interference due to quinones and polyphenols, which are oxidized to quinones, is not avoided by this modification.

If 1,4-dinitrobenzene is used, a red color develops. The limits of identification were not determined for this reagent; they seem to depend on the nature of the test material. It is probable that 3,4-dinitrobenzoic acid may also be used as the reagent, because it has been employed (*6*) for the chromatographic detection of reducing sugars.

Procedure. The test is conducted in a micro test tube. One drop of the aqueous or benzene solution of the test material is treated with 1 drop each of 25% sodium carbonate solution, 4% formaldehyde, and a 5% solution of 1,2-dinitrobenzene in benzene. The mixture is shaken and placed in a boiling water bath. The shaking is repeated intermittently. A more or less intense violet color appears in 1 to 4 minutes, depending on the quantity of catalytically active material present. A blank comparison test is recommended.

These amounts were detected:

Compound	γ	Formula
Diacetyl	0.05	$CH_3COCOCH_3$
Benzil	2	$C_6H_5COCOC_6H_5$
Furil	0.2	
Phenanthraquinone	0.002	
3-Nitrophenanthraquinone	0.002	
Ninhydrin	0.5	
Isatin	30	
2-Methyl-1,4-naphthaquinone (vitamin K_3)	0.01	
Sodium 1,2-naphthaquinone-4-sulfonate	0.5	
Anthraquinone	0.05	
Sodium anthraquinone-2-sulfonate	0.5	
Sodium rhodizonate	0.5	

Dehydroascorbic acid, *p*-benzoquinone, anthraquinone disulfonic acid, and chloranil gave strong responses. The fact that vitamin K_3 catalyzes the reaction makes it probable that vitamins K_1 and K_2, which also contain a naphthaquinone nucleus, would function in this manner. Sodium dehydrotartrate showed no catalytic effect despite the fact that it is a 1,2-diketone.

The sensitive detectability of 1,2-diketones and quinones, through their catalytic activity in the formaldehyde–1,2-dinitrobenzene system, makes possible new tests for anthracene, phenanthrene, and inositol, because these compounds are easily converted into corresponding catalytically active compounds.

DETECTION OF ANTHRACENE AND PHENANTHRENE

Anthracene and phenanthrene are converted into anthraquinone and phenanthraquinone, respectively, by evaporation with concentrated nitric acid. These products react satisfactorily with the reagent mixture, if the reagent is precipitated in a surface-rich form from its benzene solution by evaporation of the solvent.

Procedure. One drop of the benzene solution of the sample is evaporated to dryness in a micro test tube. A drop of concentrated nitric acid is added and the evaporation is repeated. A drop or two of benzene is added to the evaporation residue, and the procedure for 1,2-diketones and quinones is then followed. The depth of the color indicates the quantity of anthracene or phenanthrene involved.

Limit of identification is 2 γ of anthracene; or 3 γ of phenanthrene.

Because nitric acid merely nitrates naphthalene, this test provides a means of detecting anthracene in naphthalene if a comparison is run with pure naphthalene.

DETECTION OF INOSITOL

As shown above, the formaldehyde–1,2-dinitrobenzene reaction is catalyzed by sodium rhodizonate or rhodizonic acid. Although sodium rhodizonate is readily oxidized, by itself it has no action on 1,2-dinitrobenzene because it is not a hydrogen donor. Inositol is converted by concentrated nitric acid into rhodizonic acid (*4*) along with other aliphatic cyclic polyketones. A new test for inositol has been developed from these facts. This test is far more sensitive than the tests previously used, which are based on the production of colored alkali earth salts of rhodizonic acid (*5*).

Procedure. A drop of the aqueous solution to be tested for inositol is evaporated to dryness in a micro test tube. One drop of concentrated nitric acid is then added and the evaporation is repeated to remove the unused nitric acid. The residue is then treated according to the procedure for diketones and quinones.

Limit of identification is 5 γ of inositol.

The foregoing test may not be employed directly if reducing sugars are present, even though the sugars are partially converted to oxalic acid by evaporation with concentrated nitric acid. Consequently, a test for reducing sugars must be made prior to the evaporation. This is done by adding a drop of the benzene solution of 1,2-dinitrobenzene to the sodium carbonate test solution and warming. If reducing sugars are present, a violet color develops. If inositol is to be detected in the presence of reducing sugars or ascorbic acid, these can be quantitatively oxidized by means of alkali hypohalogenite which does not affect inositol.

Procedure. The test is conducted in a micro test tube. A drop of the aqueous test solution is treated with 1 drop of strong bromine water and 1 drop of 0.5*N* sodium hydroxide. The mixture is warmed for 1 to 2 minutes in a water bath. The excess hypobromite is then decomposed by adding a drop of 10% hydrogen peroxide solution and evaporating to dryness. The evaporation residue is then treated as described for the detection of inositol.

This procedure satisfactorily revealed 10 γ of inositol in the presence of 1000 γ of glucose.

ACKNOWLEDGMENT

Ernesto Silva collaborated in working out the test for inositol. The support of the Conselho Nacional de Pequisas is also gratefully acknowledged.

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Photometric Determination of Boron in Titanium and Its Alloys

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A method was desired which would incorporate the precision of a photometric method into the determination of small amounts of boron in titanium. Application of the carminic acid method for boron requires complete elimination of the titanium. Conditions have been found under which this may be done by cation exchange removal of the peroxy-titanium(IV) complex. Boron is determined on the residue from a neutralized and evaporated aliquot of the column effluent. Vanadium is the only interfering element that has been encountered. This interference is negligible if the alloy contains less than 0.3% vanadium with 0.005 to 0.10% boron, or if not more than 10 times as much vanadium as boron is present in the higher ranges.

IN A method for the determination of boron in titanium and its alloys, recently published by Norwitz and Codell (*6*), the sample, dissolved in hydrochloric acid, is passed through a cation exchange column to remove most of the titanium. The remainder is precipitated by boiling with calcium carbonate, after which boric acid is determined by titration with a standard base in the presence of mannitol. It occurred to the authors that the method might be made more precise for small quantities of boron if a photometric determination, such as that with carminic acid (*2*, *7*), could be substituted for the titration.

Preliminary experiments revealed that titanium in quadrivalent form interferes strongly with the carminic acid test for boron. Absorption spectra for the complexes, presented in Figure 1, illustrate the extent of this interference. As attempts to keep

the titanium in trivalent state failed to eliminate the difficulty, it became necessary to find a way of removing practically all of the titanium prior to the boron determination. The calcium carbonate digestion was discarded for several reasons, including contamination from glassware when the quantity of boron is low, coprecipitation of borate when the quantity is high, and the difficulty of obtaining a perfectly clear filtrate.

ION EXCHANGE BEHAVIOR OF TITANIUM SOLUTIONS

The experience of Norwitz and Codell, that titanium(IV) chloride in dilute hydrochloric acid solution is not retained completely on a cation exchange column, was confirmed. [After the work reported herein had been completed, the authors were informed of the work of Newstead (*5*), who was able to remove titanium satisfactorily from a dilute acid solution. Newstead passed the solution through a resin of finer particle size and at a slower flow rate. It is possible that a trace of titanium coming through would cause less interference in the quinalizarin-water titration which he employed than in the carminic acid photometric method.]

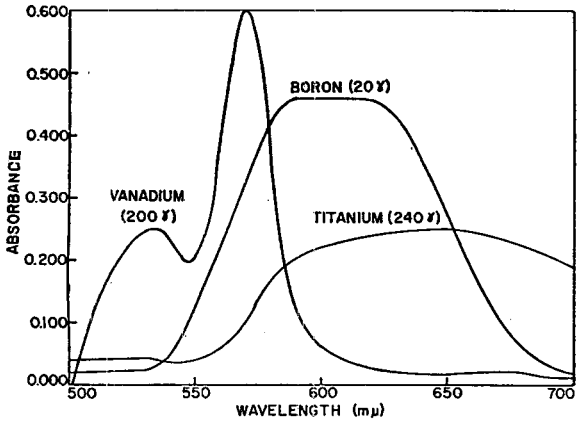

Figure 1. Absorption spectra of carminic acid complexes

The same was found true of sulfate systems. From dilute sulfuric acid solution neither titanous sulfate, nor the titanic salt prepared from it by careful oxidation with nitric acid or hydrogen peroxide, was held quantitatively by 20- to 50-mesh resin. Fortunately, however, when a clear titanous sulfate solution in 0.05 to 0.1*N* sulfuric acid is treated with a sufficient excess of hydrogen peroxide, the resulting peroxy complex can be retained completely on Dowex 50. The key to a successful separation appears to be the exclusion of any suspended or colloidal titanium dioxide, which passes readily through the column.

The peroxy-titanium complex has been interpreted variously as an anion (*4*) and as a cation (*1*). That the latter is far more probable is indicated by its retention on Dowex 50 and its failure to be held on the strongly basic anion exchange resins Dowex 1 or 2.

When it had been established that the ion exchange separation of titanium and boron is feasible, the remainder of the investigation resolved itself into a search for the conditions under which a sample can be dissolved and carried through to the final determination without contamination or losses of boron. Trial and error tests finally resulted in the procedure described below. Although it probably offers little timesaving over the titration method, it appears to yield reproducible results. The method as described covers samples containing from 0.005 to 0.10% boron; larger percentages may be determined readily by decreasing the sample weights or taking smaller aliquots.

Table I. Determination of Boron in Titanium Metal Samples

Sample	Boron Found, %	Average, %	Spectrographic Average, %
WA-39	0.0149 0.0159 0.0159	0.0156	0.0166
WA-41	0.080 0.080 0.083 0.085 0.081	0.082	0.067
Commercial titanium			
Brand I	0.0051	...	...
Brand II	0.0031 0.0023	0.0027	...

PROCEDURE

Reagents. A standard boric acid solution was prepared by dissolving 0.5716 gram of recrystallized and dried boric acid in distilled water, diluting to 1 liter, and rediluting 10 ml. to 100 ml. as needed. The final solution contains 10 γ of boron per milliliter. Approximately 5*N* sodium hydroxide solution was prepared by dissolving 200 grams of ACS grade pellets in water to make 1 liter, in a polyethylene bottle. Carminic acid solution was prepared by dissolving 0.250 gram of a product obtained from the Eastman Kodak Co. in 500 ml. of concentrated sulfuric acid.

A wash solution containing 2 ml. of 30% hydrogen peroxide and 1 ml. of 1 to 4 sulfuric acid in 100 ml. of distilled water was prepared as needed.

Other reagents were of C.P. or reagent grade. The ion exchange resin used was Dowex 50-X8, a sulfonated polystyrene, in the form of 20- to 50-mesh beads.

Apparatus. Titanium samples were dissolved in a 100-ml. Vycor flask connected by a Vycor joint with a water-cooled Vycor reflux condenser. Boron-free glass (Pyrex 74280) should also be suitable for this equipment.

The ion exchange column and back-washing assembly were arranged as shown in Figure 2.

The column was packed in the usual manner except that plugs of acid-washed glass wool (soaked in dilute hydrochloric acid for several days) were placed above and below the resin bed. If the resin is not already in the hydrogen form, the column is washed with about 50 ml. of 1 to 4 sulfuric acid, followed by about 200 ml. of water.

Absorbance measurements were made on a Beckman DU spectrophotometer, using 1.00-cm. Corex cells. The absorption spectra were recorded on a Cary Model 11 MS spectrophotometer.

Analysis of Samples. Weigh 1.0 gram of the titanium metal sample to the nearest milligram and place it in the Vycor flask. Add 10.0 ml. of 1 to 4 sulfuric acid. Connect the flask with the reflux condenser, lubricating the joint with a drop of 1 to 4 sulfuric acid. Drop a number of small pieces of dry ice through the condenser, to displace the air in the flask and tube with carbon dioxide. To keep air out of contact with the hot solution during the dissolving process, affix a 2.5-inch short-stemmed funnel to the top of the reflux condenser by means of rubber tape and keep the funnel filled with dry ice. (A small piece of wire gauze forced to fit the bottom of the funnel may be used to support the dry ice.) Heat the solution at a slow boil for 5 to 6 hours or until all the titanium has dissolved. Allow the flask to cool to room temperature. Wash down the condenser with a mixture of 5 ml. of 30% hydrogen peroxide and 5 ml. of water, and rinse the joint with a little additional diluted peroxide solution. Transfer the solution to a 50-ml. volumetric flask, taking care to avoid transferring any flakes of solid titanium dioxide which may have formed. Rinse the flask with alternate portions of dilute peroxide solution and distilled water until the total volume is almost 50 ml. Allow the mixture to cool to room temperature and make to volume.

Prewash the ion exchange column with 100 ml. of the wash solution and leave the column nearly filled with this liquid. With the stopcock on the separatory funnel closed, pipet in a 10.00-ml. aliquot of the titanium solution, add 2 ml. of 30% hydrogen peroxide plus 18 ml. of water, and swirl to mix. Beneath the column place a 100-ml. volumetric flask containing a few drops of 30% hydrogen peroxide. This is to ensure that if any titanium comes through the column it will be revealed by the appearance of a yellow color. Partially open the stopcock on the separatory funnel and start collecting the effluent at the rate of 1 drop every 2 seconds. Just as the last of the titanium solution leaves the separatory funnel, wash down the sides of the funnel with several milliliters of wash solution. Repeat this step twice

more, then add about 70 ml. of wash solution. Collect the effluent to a total volume of 100 ml. and mix.

Pipet an aliquot of the solution, containing from 2 to 20 γ of boron, into a platinum (or nickel) crucible. Using a polyethylene dropper, add 23 drops (1 ml.) of 5N sodium hydroxide solution and immediately cover the crucible with a platinum cover. With larger aliquots—i.e., 20 ml.—be sure that enough sodium hydroxide solution has been added to render the solution in the crucible strongly [illegible] carefully on a steam bath until decomposition of the hydrogen peroxide is complete. Rinse the cover with a little distilled water, catching this in the crucible, and evaporate the solution to dryness on a steam bath. This evaporation must take place in a strongly basic medium, as some boron would be lost from acidic solution (*3*, *7*). After solid material has coated the surface of the solution, finish the drying in an oven at 120° to 140° C. It is important that only a dry residue remain. Add 2.0 ml. of water to the crucible, stir with a glass rod until all of the residue has gone into solution, then transfer as much as possible to a 25-ml. graduated cylinder. Immerse the lower end of the cylinder in an ice-water slurry. Chill the platinum crucible momentarily and rinse it with about 2 ml. of concentrated sulfuric acid. While swirling the cylinder, slowly transfer the washings from the crucible. Rinse the crucible once more and transfer the washings in the same manner. Remove the cylinder from the cooling bath. Continue rinsing the crucible with 2-ml. portions of acid until the total volume in the cylinder is about 11 ml. After allowing cylinder and contents to come to room temperature, carefully adjust the volume to 12.0 ml. with concentrated sulfuric acid.

Pipet 2.00 ml. of the standard boron solution (20 γ of boron) into a separate cylinder. Chill in an ice-water slurry and add 10 ml. of concentrated sulfuric acid with swirling. Add the first 2 to 4 ml. very cautiously. Make to 12.0 ml. as before, after adjusting to room temperature. Prepare also a blank containing 2.00 ml. of distilled water. Add 10.0 ml. of carminic acid reagent to each cylinder and mix. Allow the mixture to stand at room temperature for 45 minutes. Determine the absorbance at 600 mμ, using the reagent blank for the reference solution.

Calculate the per cent boron in the titanium sample as follows:

$$\% B = \frac{\text{absorbance of sample} \times 20}{\text{absorbance of standard} \times \text{grams of sample in final solution} \times 10{,}000}$$

Titanium also forms a colored complex with carminic acid which interferes strongly with the determination of boron (see Figure 2). If the presence of titanium is suspected—for example, if the effluent has a slight yellow color—absorption due to the titanium–carminic acid complex should be corrected for by determining the absorbance at 710 mμ and employing the following equation:

$$B = 1.032X - 1.335Y$$

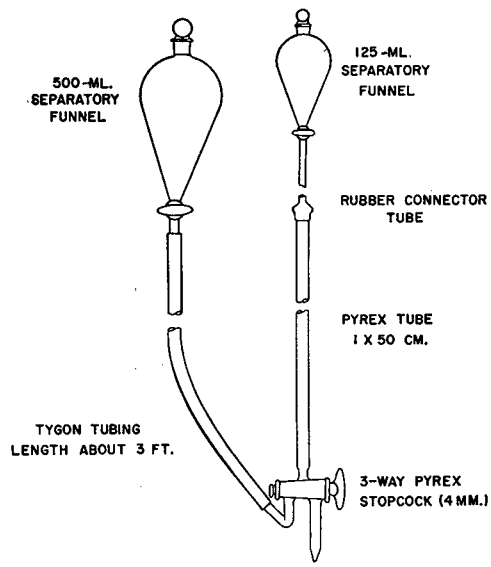

Figure 2. Ion exchange column and backwashing assembly

where B = absorbance at 600 mμ due to boron
X = absorbance reading at 600 mμ
Y = absorbance reading at 710 mμ

This equation is based upon the ratio of readings at 600 and 710 mμ for each of the individual complexes.

Notes. Although very little solid titanium dioxide forms when the sample is dissolved in the manner described, care must be taken to avoid transferring any solid from the Vycor flask to the volumetric flask. If suspended titanium dioxide is allowed to reach the ion exchange column, the finely divided particles tend to filter through the resin bed and into the effluent. Some of this titanium will dissolve and interfere strongly with the carminic acid determination of boron.

The acid concentrations of the titanium solution and of the wash solution are critical. The diluted titanium solution which is passed through the column is about 0.07N in sulfuric acid and the concentration of the wash liquid should be the same. If the acid concentration is much higher, not all of the titanium is retained on the resin. It is not possible to neutralize excess acidity, since the cation of any base used will be replaced by hydrogen ions from the resin to give the original acid concentration. An appreciable excess of hydrogen peroxide is needed to minimize dissociation of the peroxy titanium complex.

Table II. Recoveries of Boron Added to Titanium Metal

Sample	Boron Found, %	Average, %	Calcd. Average, %
Brand II	0.0031 0.0023	0.0027	...
+0.005% B	0.0057 0.0054	0.0055	0.0077
+0.05% B	0.052 0.053	0.052	0.0527
+0.10% B	0.101 0.103	0.102	0.1027

Boron added as boric acid.
Sample, Brand II commercial titanium.

Color development of the boron–carminic acid complex is considerably affected by variations in acid concentration and by the particular batch of carminic acid used to make up the carminic acid reagent solution. For this reason it is better to run a standard with each series of samples than to rely upon a standard curve. The absorbance of the complex was found to follow Beer's law for all concentrations tried, when the same reagents were used.

The ion exchange column is regenerated by back-washing alternately with 100-ml. portions of 1 to 4 sulfuric acid and water, each containing several drops of 30% hydrogen peroxide. In back-washing with water the flow should be stopped for a moment after about 20 ml. of water has passed through, to permit the resin bed to settle to the bottom of the column. This greatly increases the washing efficiency. The pause may be repeated after another 20 ml. of water has passed through. Three or four acid-water cycles are usually sufficient to remove the last traces of titanium, as evidenced by the disappearance of the titanium–hydrogen peroxide color.

EXPERIMENTAL RESULTS

No titanium samples containing accurately known amounts of boron were available. However, two samples of the metal which had been analyzed for boron by several different laboratories were also analyzed by this procedure, with the results shown in Table I. These samples were supplied by the Metallurgical Advisory Committee on Titanium, Panel on Methods of Analysis. Also included in Table I are the spectroscopic results on these same samples obtained by J. H. Enns of the University of Michigan. The two sets of results differ somewhat. At this concentration

level the method described seems to be precise. The results obtained on two samples of commercial titanium are also shown in this table. These happened to be low in boron.

As a check on the precision and accuracy of the method, several recovery experiments were carried out by adding known amounts of boric acid to samples of low-boron titanium (Brand II). Total boron was then determined by the procedure as described (Table II). At the 0.05 and 0.10% levels the recoveries are good. At the 0.005% level the relative error is rather large, but even here the results should be adequate for most practical purposes.

The probable accuracy of the method is ±0.002% for samples containing 0.5 to 0.1% boron.

INTERFERING ELEMENTS

Most of the common elements in reasonable quantities are known to cause no interference in the carminic acid method for boron (*2*). In amounts that are likely to occur in titanium alloys, the following do not interfere, as shown by tests in this laboratory: chromium, aluminum, zirconium, nickel, iron, tungsten, columbium, tantalum, and silicon.

Vanadium represents a possible serious interference, which apparently has not been reported previously. The absorption spectra of Figure 1 illustrate the magnitude of the interference at various wave lengths, when 10 times as much vanadium as boron is present. These curves were obtained by treating the indicated quantity of the elements as in the color development step of the procedure and reading against a reagent blank as the reference solution. The vanadium–carminic acid complex does not exhibit such a sharp absorption peak when read against water or sulfuric acid, but the shift in the absorption curve as measured against the blank produces the spectrum shown. This system does not follow Beer's law, and in practice the interference of vanadium becomes negligible when less than 0.3% is present in the sample. Larger quantities may be tolerated if the vanadium content is not more than ten times the boron content. Vanadium is not retained at all by the ion exchange resin under the conditions specified, so some other method of removing it would be necessary if too much were present.

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Moisture Permeability of Glove Materials for Controlled-Atmosphere Boxes

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The moisture permeability of glove materials has been found to be the limiting factor in the maintenance of dry atmosphere boxes. A comparison of available materials for this use has shown the superiority of butyl rubber over all other materials tested.

ATTEMPTS to maintain extremely dry atmospheres in glove boxes for handling moisture-sensitive materials have met with repeated failure in spite of rigorous sealing techniques and free use of desiccants. Experience has indicated that a major path by which moisture enters such boxes is through the gloves, which form a significant part of the inside area of a glove box (on the order of 3000 sq. cm. per glove). In the work reported here, the moisture permeability of samples from commercially available gloves, and of other materials which showed promise or were of interest, was measured.

A search of available literature revealed that the moisture permeability of natural rubber films is dependent upon many factors, such as the age of purified latex before vulcanization (*3*), the kind and amount of filler used (*4*), plasticization and calendering (*3*), and vulcanization conditions (*1*). From the work of Corwin and Karr (*2*), who demonstrated the superiority of butyl rubber tubing in equipment for organic carbon-hydrogen analyses, it was concluded that this type of material might also be a superior material for gloves. The work of Thomas and coworkers (*5*) also suggested the superiority of butyl rubber. As no butyl rubber gloves were commercially available, sample sheets with thicknesses equivalent to other glove materials were obtained for testing. Other natural and synthetic materials were also tested for comparison.

APPARATUS AND PROCEDURE

Three conditions of relative humidity and temperature were selected for the permeability tests: 24° C. and 58% relative humidity (corresponding to normal conditions in a glove when the box is not in use), 24° C. and 100% humidity (corresponding to the atmosphere on the exposed side of the glove just after use), and 37° C. and 100% humidity (corresponding to the conditions within a glove when it is actually in use). All materials were subjected to the first two sets of conditions, and materials of special interest were further subjected to the last set of conditions.

The apparatus was, in part, modeled after similar equipment used by Florin (*4*). The atmosphere box and sample holder are shown diagrammatically in Figure 1 as they were arranged for elevated temperature and humidity conditions. For measurements at 24° C. and elevated humidity, the heat lamp was disconnected, but the water tray (with cellulose sponge maze to increase evaporation surface area) was left in place and the atmosphere box was kept closed. At 24° C. and 58% relative humidity (normal laboratory conditions) the water tray was removed, and one side of the atmosphere box was opened to the laboratory, so that room air could be circulated over the sample by an external fan.

The temperature and humidity within the atmosphere box were observed with a wet-dry bulb thermometer. Normal laboratory conditions were also monitored with the aid of a recording Hythergraph. Throughout each test the temperature in the box was observed to be constant within ±1° C., while the relative humidity at 58% was constant within ±2%. It was not possible to maintain exactly 100% relative humidity, because the atmosphere box was neither sealed nor insulated.

The test sample consisted of a disk 25 cm. in diameter, of which a test area of 346 sq. cm. was exposed to the atmosphere in the box. Each sample was smoothed over the face of the sample holder, and was held in place by the Teflon O-ring and the retaining ring, shown in Figure 1. Precautions were taken to prevent leaks caused by channeling under the edge of the sample. The sample was under no tension, as indicated by a tendency to balloon slightly when argon was swept through the system.

Table I. Permeability of Materials Tested[a]

Sample No.[b]	Thickness, Mm.	Density, G./Cc.	Temp., ° C.	% Relative Humidity	Total Time, Hr.	Total H_2O collected, Mg.	Permeability, γ/Hr./Sq. Cm.
1	0.81	1.21	24	58	112.7	13.4	0.34
			24	100	112.5	19.1	0.49
			37	100	46.7	24.9	1.54
2	0.74	1.06	24	58	43.7	7.4	0.49
			24	100	39.8	8.6	0.62
			37	100	39.9	23.6	1.71
3	1.09	1.23	24	58	47.7	49.8	4.8
			24	100	49.7	103.0	6.3
			37	100	23.7	177.0	21.6
4	0.64	1.21	24	58	23.6	50.4	6.2
			24	100	23.9	99.7	12.1
			37	100	24.8	506.9	59.1
5	0.74	1.25	24	58	39.7	121.6	8.8
			24	100	28.3	162.7	16.6
6	0.43	1.32	24	58	8.7	24.2	8.1
			24	100	32.2	190.1	17.1
7	0.64	1.14	24	58	63.7	224.6	10.2
			24	100	87.6	638.9	20.8

[a] Order of analysis was 5, 4, 6, 3, 7, 2, 1. All room temperature studies were made first, followed by elevated temperature studies.
[b] 1. Butyl rubber sheeting No. 6948, 45–50 Duro.
2. Butyl rubber sheeting No. 6935, 30–35 Duro.
3. Synthetic rubber A, sample cut from elbow-length gauntlet glove designed for handling corrosive materials.
4. Synthetic rubber A, sample cut from shoulder-length gauntlet glove designed for use in glove boxes.
5. Natural cloth-backed rubber, white sample cut from shoulder-length gauntlet glove designed for handling corrosive materials.
6. Synthetic rubber B, shoulder-length gauntlet glove with universal hand designed for glove box.
7. Synthetic rubber C, sample cut from elbow-length gauntlet glove designed for handling corrosive materials.

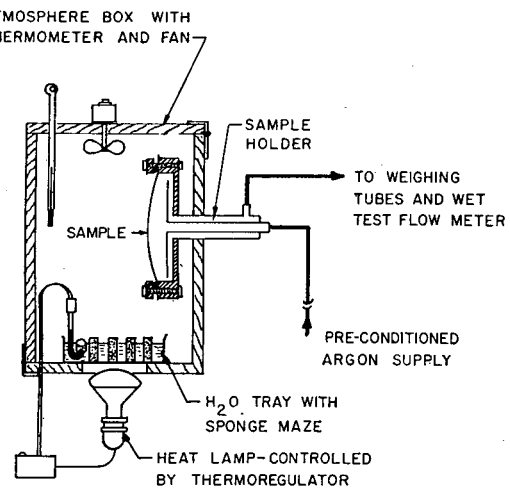

Figure 1. Permeability apparatus

Desired temperature and humidity conditions were established in the atmosphere box before the sample holder was mounted and connected into the system. "Preconditioned" argon was then allowed to flow through the system for several hours prior to determination. The permeability of a sample was measured by determining the gain in weight of magnesium perchlorate–charged U-tubes for a timed period and metered argon flow. The flow rate during flushing and measurement was 4 to 6 liters per hour. Moisture recovery did not appear to be influenced greatly by flow rate, unless the rate was extremely low. Duplicate determinations were made for most samples; additional determinations for samples of special interest were made to assure attainment of apparent equilibrium. Whenever possible, a determination was continued until a significantly weighable amount of water had been collected.

Blank determinations were made by bypassing the sample holder.

DISCUSSION AND RESULTS

The determination of weight changes in tubes filled with "anhydrous" magnesium perchlorate cannot be considered a quantitative measure of the water content of gases whose moisture content approaches or is less than that of the desiccant.

Willard and Smith (*6*) found the residual water at equilibrium over anhydrous magnesium perchlorate at 25° C. to be 5×10^{-4} mg. per liter, and over magnesium perchlorate trihydrate to be 2×10^{-3} mg. per liter. As the tank argon, as available in this laboratory, was found to be so consistently dry that the dew point was below the estimated limit of sensitivity of an Alnor Dewpointer (130° A., corresponding to a moisture content of less than 1×10^{-5} mg. per liter), the gas was "preconditioned" by passing through two 25-cm. towers packed with magnesium perchlorate followed by a cold trap of dry ice in acetone. By this procedure the moisture content of the argon sweep gas was increased to a level which gave a positive blank in the weighing tubes. Continuous use of the preconditioning train resulted in a gradual depletion of the available moisture in the preconditioning train. At the start of the experiment the blank was +1.6 mg. in 65 hours with freshly filled towers; after the towers had been used for 73 days, the blank was −2.4 mg. in 23 hours. These blank values are insignificant for the purpose of relative comparison. However, as the body temperature studies (37° C.) were made after all room temperature studies (24° C.) had been completed, it is probable that the 37° C. permeability tests were affected by negative blanks.

For these reasons the tabulated results have not been corrected for the presence or absence of residual moisture in the sweep gas. Consequently, the results are not absolute, although they provide an adequate basis for relative comparison.

Experimental results along with descriptions of the materials sampled and analyzed are given in Table I. Experimentally determined thickness and density values are provided to give a more complete description. Tabulated thicknesses are average values for measurements made with micrometer calipers at various places on the sample tested. Density values were calculated from the mean thicknesses and weights of test areas.

Time of analysis and weight of water collected are accumulated totals of all determinations for each sample. Individual determinations varied from 4 to 65 hours. The accumulated totals were used to calculate permeabilities because they were thought to yield a more valid measure.

CONCLUSIONS

The results obtained for the samples tested indicate that butyl rubber has a moisture permeability about one tenth that of the next best material tested. None of the parameters mentioned in the introduction was known or controlled for this experiment; consequently, the results presented should not be considered as applicable to other materials which appear similar. No correction has been considered for variations in sample thickness, although permeability would be expected to follow an inverse relationship to thickness.

ACKNOWLEDGMENT

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Polarographic Determination of Cobalt in Presence of Nickel

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A new modification of the Kolthoff-Watters procedure for the determination of traces of cobalt in the presence of nickel involves oxidation of cobalt(II) in an ammoniacal ammonium chloride solution of the sample to the +3 state with excess permanganate, followed by the destruction of the excess permanganate with excess hydroxylammonium sulfate. The results are accurate to within ±5%, and a determination is easily completed in 15 minutes or less.

ALTHOUGH much effort has been devoted to the development of procedures for the analysis of mixtures of nickel and cobalt, very few satisfactory methods have been proposed for the determination of traces of cobalt in the presence of a large excess of nickel or zinc. The best of these are doubtless the ones described by Kolthoff and Watters (*1*, *2*, *7*, *8*), both of which involve the oxidation of cobalt to the +3 state and the measurement of the polarographic diffusion of the cobaltic complex.

Such a procedure is necessary in any polarographic method for two reasons. First, the nickel wave precedes that of cobalt(II) in nearly every known supporting electrolyte, so that it would be virtually impossible to measure the very small diffusion current of the cobaltous wave. Secondly, no medium is known in which cobalt(II) gives a wave at a potential sufficiently different from that of zinc to permit these elements to be distinguished, and zinc is of course a ubiquitous contaminant of nickel salts.

Probably the more elegant and useful of the methods proposed by Kolthoff and Watters, because of its greater freedom from interferences, is that in which the cobalt is oxidized by sodium perborate in an ammoniacal solution (*7*, *8*). Unfortunately, the excess perborate has to be removed by boiling the solution (under reflux to prevent excessive loss of ammonia), which must then be cooled before its polarogram can be recorded. The time consumed in these operations makes the procedure rather too lengthy for the routine laboratory.

The method described in this paper is very similar to that of Kolthoff and Watters, but uses potassium permanganate instead of sodium perborate. The excess permanganate is instantly destroyed even at room temperature on addition of an excess of hydroxylammonium sulfate. This eliminates the necessity of refluxing and cooling the solution. The excess hydroxylamine does not reduce the cobalt(III) complex at a measurable rate at room temperature.

EXPERIMENTAL

All polarographic measurements were made with a pen-and-ink recording polarograph (*5*) and an entirely conventional dropping mercury electrode assembly. The modified H-cell (*6*), equipped with a sintered borosilicate glass gas dispersion cylinder to permit rapid deaeration, was secured from E. H. Sargent and Co. (S-29438). All polarograms were recorded at 25.0° ± 0.5°.

A stock solution of cobaltous sulfate was prepared from the reagent grade salt and standardized by coulometry at controlled potential (*3*, *4*). An accurately known volume of the cobalt solution was added to about 75 ml. of 1*M* ammonia–1*M* ammonium chloride in a double-diaphragm cell for controlled potential electrolysis (*4*). Dissolved air was removed by a rapid stream of prepurified nitrogen; then 25 ml. of mercury was added and the solution was electrolyzed at −1.10 volts *vs.* S.C.E., using a potentiostat (Analytical Instruments, Inc., Bristol, Conn.) to maintain a constant electrode potential. This served to remove traces of copper and nickel, and also to reduce any cobalt(III) formed by reaction with dissolved oxygen. A current integrator (Analytical Instruments, Inc.) was then connected into the electrolysis circuit and the potentiostat was readjusted to keep the working electrode potential constant at −1.45 volts *vs.* S.C.E. At this potential cobalt(II) is completely reduced to the metal, and the difference between the initial and final coulometer readings gave the number of milliequivalents of cobalt directly.

The results of seven such experiments are shown in Table I.

For the reasons described above, this procedure actually gives the sum of cobalt and zinc, but the percentage of the latter element present could hardly have been significant in this work. When the amount of zinc present can be neglected, this procedure would appear to be one of the most accurate and convenient available for the standardization of a cobalt solution.

RECOMMENDED PROCEDURE

Weigh 2.50 grams of the nickel salt to be analyzed into each of two 100-ml. volumetric flasks, and to each add about 50 ml. of water, 2.5 ± 0.5 grams of ammonium chloride, and about 10 ml. of concentrated ammonia. To one of the two flasks add 2 ml. of saturated potassium permanganate solution and let stand for a minute or two to ensure complete oxidation of the cobalt. During this period a brown turbidity of manganese dioxide will form slowly: The appearance of a large precipitate immediately after the permanganate is added indicates the presence of a considerable amount of arsenic, antimony, chromium, or manganese. In that event, add more permanganate until the solution is definitely purple. Finally add 2 ml. of saturated hydroxylammonium sulfate and 1 ml. of 0.2% Triton X-100 (Rohm & Haas Co., Philadelphia) to each flask, and dilute each solution to the mark. Allow a few seconds for the excess permanganate and manganese dioxide in the oxidized solution to react completely and for vigorous evolution of nitrogen to cease, then stopper and shake thoroughly.

Transfer a portion of the oxidized solution to a polarographic cell, deaerate it with hydrogen or nitrogen, and record its polarogram from −0.2 to −0.8 volt *vs.* S.C.E.

Table I. Coulometric Standardization

$CoSO_4$ Used, Ml.	Millifaraday Consumed	Millifaraday/Ml. Normality
9.891	2.054_3	0.2076_9
	2.061_7	0.2084_4
9.998	2.064_3	0.2064_7
	2.074_1	0.2074_5
	2.071_7	0.2072_1
	2.071_7	0.2072_1
14.900	3.092_4	0.2075_5
		Mean 0.2074 ± 0.0004

The resulting curve will resemble curve *B* of Figure 1, which was secured during the analysis of a sample of reagent grade nickel chloride. The half-wave potential of the cobalt(III) wave is approximately −0.40 volt *vs.* S.C.E. This is close to the mean of the values reported in the literature for the waves of the hexammino- and aquopentammino-cobalt(III) ions. No doubt it is a mixture of these which results from the oxidation with permanganate and is responsible for the abnormally small slope of the wave.

Discard this solution and replace it with a portion of the solution which had not been treated with permanganate. Deaerate this and record its polarogram under exactly the same conditions and with the same polarograph settings that were used in recording the first polarogram. The resulting polarogram will resemble

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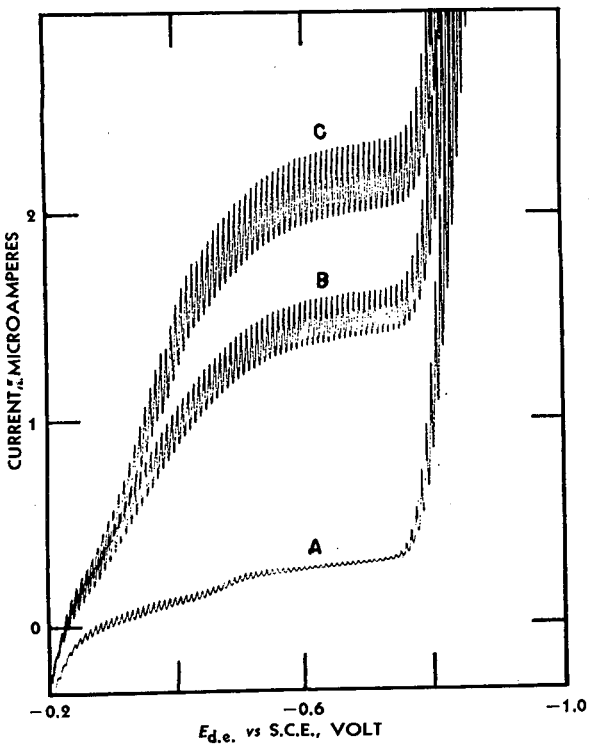

Figure 1. Polarograms of reagent grade nickel chloride treated by recommended procedure

A. **Without permanganate**
B. **After oxidation with permanganate**
C. **After addition of known amount of cobalt and oxidation with permanganate**

Table II. Proportionality between Cobalt Concentration and Diffusion Current

Mg. Co/100 Ml.	Millimole Co/Liter	i_d, μa.	i_d/C
0.153	0.0260	0.110	4.23
0.307	0.0521	0.217	4.16
0.615	0.1042	0.420	4.03
1.23	0.208	0.866	4.16
1.84	0.312	1.28	4.10
2.46	0.416	1.74	4.19
3.07	0.521	2.06	3.95
3.69	0.625	2.54	4.06
6.15	1.042	4.27	4.09
18.4	3.12	12.65	4.05
36.9	6.25	25.5	4.10
61.5	10.42	44.1	4.23
184	31.2	123.9	3.97
		Mean	4.11 ± 0.07

curve *A* in Figure 1. It always contains a small wave at about −0.5 volt *vs.* S.C.E. This is due to the reduction of the cuprous ammonia complex formed by the reaction between the copper contained in the sample and the excess hydroxylamine. It is evident that a single polarogram of the oxidized solution would both give an erroneously high value for the cobalt concentration and fail to reveal that an error was being made.

Measure the vertical distance between the two curves at a potential on the plateau of the cobalt wave, roughly −0.70 volt *vs.* S.C.E. Compare the diffusion current thus measured with the values obtained when solutions containing known amounts of cobalt are treated in the same way.

DATA AND DISCUSSION

Table II shows some of the results obtained when known cobalt solutions were treated by the recommended procedure. The diffusion current is closely proportional to the cobalt concentration over a very wide range.

The final solutions are about 0.5*M* in ammonium chloride and 1*M* in ammonia. Decreasing the ammonium chloride concentration to 0.2*M* gave much less reproducible results. The diffusion current was always lower than the value predicted from Table II, and the ratio of the diffusion current to the cobalt concentration decreased considerably with increasing cobalt concentration. These phenomena became even more pronounced if the ammonium chloride was omitted altogether, or if its addition was deferred until after the addition of the hydroxylammonium sulfate. In the absence of a sufficient concentration of ammonium ion—i.e., at pH values much higher than those attained in the recommended procedure—the oxidation of cobalt(II) by permanganate apparently proceeds with considerable difficulty and fails to reach completion even after a considerable length of time. Whereas a solution containing the recommended concentration of ammonium ion becomes cherry red almost immediately when permanganate is added, and slowly deposits manganese dioxide, a solution containing little or no ammonium ion remains purple long after the permanganate is added.

Table III. Effects of Possible Impurities in Nickel Salts on Determination of Cobalt

(Each sample contained 2.50 grams of nickel nitrate, 1.21 mg. of cobalt, and specified amount of one other element)

Substance Added, Mg.	Cobalt Found, Mg.
75 As	1.20
200 Bi	1.11
1200 Cd	1.22
100 Cr ($BaCl_2$ added)	1.24
1 Cu	1.21
10 Cu	1.26
25 Cu	1.2
10 Fe	1.22
100 Fe	0.95
30 Mn	1.21
100 Mo	0.97
200 Pb (Na_2SO_4 added)	1.24
120 Sb	1.16
120 Sn	1.18
120 U	1.15
50 V	0.0
180 W	0.0
1400 Zn	1.24

On the other hand, the wave secured in the presence of 1*M* ammonium ion is appreciably less well defined—it begins earlier and ends later, and the plateau is considerably shorter. Though a variation of about ±20% in the ammonium ion concentration has little or no effect on either the rate of the oxidation or the characteristics of the wave, a much wider deviation from the recommended value should be avoided. If a strongly acidic solution (such as that obtained on dissolving a sample of nickel metal in nitric acid) has to be analyzed, it should be nearly neutralized with sodium hydroxide rather than with ammonia before beginning the analysis.

The concentration of ammonia is much less important. No detectable error results from using even 3*M* ammonia. Care should, however, be taken to ensure that the ammonia concentration is high enough to prevent any precipitation of hydrous nickel oxide, for this might coprecipitate an appreciable amount of cobalt.

Table III illustrates the results obtained when solutions containing 2.50 grams of nickel nitrate, enough added cobalt to give a total of 1.21 mg. of this element, and the specified amounts of other elements were analyzed by the recommended procedure. Arsenic, cadmium, antimony, tin, and zinc do not interfere. Bismuth, copper, iron, manganese, and molybdenum can be tolerated in moderate amounts. [The cobalt diffusion current becomes difficult to measure precisely in the presence of a large excess of copper. Bismuth and iron interfere by coprecipitating cobalt, and molybdenum by forming a moderately insoluble cobalt(III) molybdate. The presence of a large amount of nickel, incidentally, greatly reduces the errors caused by bismuth and iron.] Relatively large amounts of chromium and lead can be handled by adding an excess of either barium chloride or

Table IV. Determinations of Cobalt in Reagent Grade Nickel Salts

Salt	Cobalt Present, % Manufacturer	Polarographic analysis
$Ni(OAc)_2$	0.016	0.149 ± 0.003
$NiCl_2$	0.10	0.096 ± 0.003
$Ni(NO_3)_2$	0.05	0.0049 ± 0.0006
$NiSO_4$	0.03	0.0140 ± 0.0002
$Ni(NH_4)_2(SO_4)_2$	0.05	0.0079 ± 0.0005
Ni(metal)	0.01	0.0065 ± 0.0009

sodium sulfate, respectively. Tungsten and vanadium must be absent.

The reproducibility attainable by the procedure described is illustrated by the fact that 13 analyses of a sample of reagent grade nickel chloride (stated by its manufacturer to contain no more than 0.10% cobalt) gave a mean cobalt content of 0.096%, with a standard deviation of ±2.6%, the extreme values being 0.092 and 0.100%.

In Table IV are given the results obtained when samples of various nickel salts were analyzed by the recommended procedure. Each value in the last column is the mean of at least three results; that given for the chloride was taken from the analyses described in the preceding paragraph, and the interesting figure for the acetate was derived from six analyses of material taken from various parts of a bottle whose seal was intact when it reached the author.

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Rapid Gravimetric Determination of Mercury in Organic Compounds

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In a rapid method for determining mercury in certain organic compounds the compound is decomposed by refluxing with hydriodic acid containing iodine. Mercury forms the HgI_4^{--} ion, which is then precipitated and weighed as cupric propylenediamine mercuriiodide, $Cupn_2HgI_4$. Compounds such as methyl mercuric hydroxide, bromide, and iodide and diphenylmercury give results which are precise but about 1% low. Part of this error is caused by incomplete precipitation.

A REASONABLY fast and accurate method is needed for determining mercury in organic compounds. Most published methods depend on oxidation of the compound, either by oxygen in a combustion tube or by such agents as sulfuric and nitric acids, ammonium persulfate, or potassium permanganate. Combustion with oxygen is long and tedious, though reliable. Some of the wet oxidation methods cannot be used in presence of halogen. A few methods depend on reduction to metallic mercury, but these generally give low results.

Iodine has long been known to attack organic mercury compounds (*4*, *5*). Hydrogen iodide, of course, is a very effective reducing agent and decomposes many substances. A combination of iodine and hydriodic acid was found to attack alkyl and aryl mercuric halides and hydroxides very rapidly, forming the stable complex ion HgI_4^{--}. The next step was to find a way of quantitatively determining mercury in the form of this ion. Spacu and Spacu (*2*) precipitate the salt $Cupn_2HgI_4$ (pn = 1,2-propanediamine) by adding cupric propylenediamine sulfate and weigh it. They claim an accuracy of 1 part in 1000 for 0.5-millimole quantities of mercury added as mercuric chloride.

To explore the possibilities of this method for organic compounds, two things were necessary—a study of the Spacu method for inorganic mercury and a study of the effectiveness of the hydriodic acid–iodine digestion.

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Spacu and Spacu Method. To 100 to 250 ml. of a solution of a mercuric salt add 2 grams of potassium iodide per 100 ml., and make weakly basic with ammonia. Heat to boiling, add an excess of a boiling concentrated solution of cupric propylenediamine sulfate, then cool to room temperature, filter, and wash the precipitate first with a solution containing 1 gram each of potassium iodide and cupric propylenediamine sulfate per liter, then with alcohol and ether. Dry in a vacuum desiccator at room temperature and weigh.

Duval and Dat Xuong (*1*) showed that drying at room temperature was unnecessary, and that the precipitate could be heated to 157° C. without decomposition. They consider this method one of the best gravimetric methods for mercury. The precipitate is easily filtered and dried, and its molecular weight, 920.2, gives a very favorable gravimetric factor.

EXPERIMENTAL

Modification of Spacu Method. The cupric propylenediamine sulfate reagent was made by mixing 1 volume of 1,2-propanediamine (Eastman practical grade, redistilled) with 5 volumes of 1*M* cupric sulfate. The question arose whether this grade of propylenediamine was sufficiently pure for the purpose, for ethylenediamine, a probable impurity, would not be removed by distillation. A quantity of propylenediamine was therefore made by converting the redistilled "practical" amine to the sulfate, recrystallizing this from aqueous methanol, and reconverting to free amine by distilling from sodium hydroxide. Reagent made from this purified amine gave the same analytical results as that made from the redistilled practical amine. Furthermore, the addition of 5% ethylenediamine to the propylenediamine had practically no effect on the analysis.

Cupric propylenediamine sulfate reagent which was 6 months old gave the same results as fresh reagent. This is in contradiction to Spacu and Spacu, who say that the reagent must be freshly mixed and should be heated before being added to the ammoniacal mercury solution. This heating was found to be unnecessary.

Not only is it unnecessary to wash the precipitate with ether,

Table I. Recoveries of Inorganic Mercury

Salt	No. of Detns.	Taken, Mmole	Found, Mmole (Arithmetic Mean)	Standard Deviation	Loss %	Loss Mmole
$HgCl_2$	7	0.1104	0.1090	0.0009	1.27	0.0014
	6	0.2752	0.2738	0.0004	0.51	0.0014
	5	0.5504	0.5499	0.0005	0.09	0.0005
$HgBr_2$	3	0.1109	0.1098	0.0003	1.00	0.0011
	11	0.2764	0.2752	0.0006	0.45	0.0012
	3	0.5528	0.5516	0.0007	0.22	0.0012

but it is potentially harmful. Ether that contained peroxide was found to discolor the precipitate, forming a red-brown substance which is probably cuprous mercuriiodide, and to make the results as much as 5% low.

The precipitate could be safely dried at 105° to 110° C. and left at 105° overnight without losing more than 0.3 to 0.5 mg.

Accuracy of Spacu Method. To test the accuracy of the method as modified, standard solutions were prepared from mercuric chloride and bromide (analytical reagent grade, once recrystallized from water) and known volumes were pipetted out and diluted to 75 ml. before addition of the reagent. The results are summarized in Table I. All were low, but not seriously so, and the precision was good. The percentage error fell with increasing amount of mercury, but the error term, in millimoles, was nearly constant. This suggests that the low results were caused by the solubility of the precipitate; experiments in which weighed amounts of dry precipitate were suspended in water and put through the motions of the procedure, then recovered and weighed, supported this view.

By increasing the amount of added potassium iodide fivefold, the accuracy was slightly increased. Chloride, nitrate, sulfate, phosphate, and acetate anions did not interfere in 100-fold excess, but phosphate and acetate interfered in higher concentrations, causing low results. At least a twofold excess of iron or aluminum could be present without causing error if citrate was added to prevent precipitation of hydrous oxides.

Procedure for Organic Compounds. Organic materials were refluxed for 30 minutes or more with a reagent made by mixing 40 grams of potassium iodide, 4 grams of iodine, and 100 ml. of 6*N* sulfuric acid. The presence of free iodine in the reagent is essential. Best results were obtained with 20 to 100 mg. of mercury and 10 ml. of reagent. Refluxing was done in a 100-ml. flask with standard-taper joint. If the unknown was a solution, not more than 5 ml. was taken; if it was a solid, it was dissolved in 5 ml. of Cellosolve (diethyl ether of ethylene glycol) before addition of the reagent. Ethylene glycol or ethers of diethylene glycol were suitable solvents.

After refluxing, the solution was diluted with water to 75 to 100 ml. in a beaker and heated to boiling. Solid sodium sulfite was carefully added to reduce the iodine color to a faint yellow. Excess of sulfite later produced insoluble cuprous mercuriiodide; therefore, any excess of sulfite was removed by adding enough potassium iodate to restore a faint yellow iodine color. Ammonia was then added to about pH 7 (by test paper), and 5 ml. of cupric propylenediamine sulfate reagent added to the boiling solution. The solution was cooled as fast as desired (the precipitate is coarsely crystalline even with rapid cooling), and filtered through a sintered-glass or porous porcelain crucible. The precipitate was washed with some 50 ml. of a solution containing 1 gram of potassium iodide and 2 ml. of cupric propylenediamine sulfate reagent in 1 liter of water, then with 25 ml. of 95% ethyl alcohol, dried at 105° C. for 15 to 30 minutes, then weighed. The crucibles were cleaned with aqua regia.

Drops of a heavy oily liquid were sometimes seen on diluting after refluxing of Cellosolve solutions. These did not interfere in the determinations.

The method was tested with several pure compounds. These were recrystallized solids except for methyl mercuric hydroxide; an aqueous solution of this compound was made from methyl mercuric iodide and silver oxide, and standardized by adding excess potassium bromide and titrating the potassium hydroxide which was liberated. This gives a very sharp end point (*3*). The results of these analyses are summarized in Tables II and III.

Good results were also obtained with methyl mercuric chloride, phenyl mercuric acetate and hydroxide, and *o*-chloromercuriphenol, using 1-hour digestion times. Five samples of diphenylmercury were also heated in sealed tubes with 10 ml. of reagent for 90 minutes at boiling water temperature; the accuracy and precision were not sensibly different from those obtained by refluxing. Probably better results could be obtained with diphenylmercury by digestion in sealed tubes at a higher temperature.

Mercurochrome, on the other hand, could not be analyzed by this method. An hour's digestion not only did not destroy all the Mercurochrome, but also produced tarry products which interfered seriously with subsequent precipitation and filtration. Tarry masses were also obtained with pharmaceutical tinctures containing phenolic compounds.

The elapsed time per determination is of the order of 2 hours. The operating time, however, is only 20 to 30 minutes.

Table II. Recoveries of Organic Mercury

Compound	No. of Detns.	Taken, Mmole	Reagent Used, Ml.	Reflux Time, Min.	Average Hg Loss %	Average Hg Loss Mmole
CH_3HgOH	8	0.3045	5	30	1.0	0.0030
	2	0.3045	5	20	0.86	0.0026
	6	0.3045	5	15	0.3	0.0009
	2	0.3045	5	10	2.5	0.0074
	3	0.3045	10	20	0.13	0.0004
CH_3HgBr	2	0.3–0.4	5	60	1.03	0.0038
CH_3HgI	4	0.2–0.5	5	30	1.28	0.0037
C_6H_5HgCl	2	2.0–2.5	10	60	0.03	0.0007
$(C_6H_5)_2Hg$	2	0.2000	5	60	3.5	0.0070
	3	0.2000	10	30	1.7	0.0034
	3	0.2000	10	240	1.5	0 30

Table III. Examples of Precision

Compound	Reflux Time, Min.	Taken, Mmole	Found, Mmole	Hg Loss, %
CH_3HgOH	15	0.3045	0.3044	0.03
			0.3041	0.12
			0.3024	0.68
			0.3024	0.68
			0.3043	0.06
			0.3039	0.19
CH_3HgBr	60	0.3579	0.3542	0.97
		0.3924	0.3881	1.09
CH_3HgI	30	0.2145	0.2110	1.66
		0.2592	0.2560	1.23
		0.2937	0.2892	1.53
		0.5053	0.5018	0.69

ACKNOWLEDGMENT

It is a pleasure to express thanks to the Ringwood Chemical Corp. and Panogen, Inc., for supplying mercury compounds used in this work.

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Self-Equilibrating Electrolytic Method for Determination of Acid Production Rates

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As a method for measuring cholinesterase activity quickly and accurately was desired, an automatic electrolytic titrator was developed. The titrator is designed to measure directly the rates of acid-producing reactions, but with appropriate modifications it may be used to measure the rates of other chemical reactions or dynamic processes such as diffusion or solution.

IN RECENT years numerous articles have described coulometric titration techniques for analyzing oxidizing or reducing compounds, ions such as hydrogen, copper, or chloride, and some organic compounds such as mercaptans. Cooke and Furman (*2*) have given an excellent review of these methods up to 1950. In most cases to date the techniques have been used to determine amounts or concentrations of these substances—i.e., batch method of analysis. In certain instances (*1, 3, 4, 8*) they have been used for continuous titrations, with automatic devices to follow changing amounts of the titrants, but the technique has not been used to follow the kinetics of chemical reactions taking place in the electrolytic cell. For this purpose the instrument described has been developed. Specifically, it has been developed to determine the rate at which cholinesterase (ChE) catalyzes the hydrolysis of acetylcholine chloride (AcCh), but with slight modifications it could be used to measure other reactions.

When studying enzyme systems it is often very difficult or impossible to determine the concentration of active enzyme present in a tissue preparation, but it is sometimes possible to measure a value that is proportional to the concentration. This value, called activity, is obtained by measuring the rate at which the enzyme acts upon its substrate under conditions which make the reaction pseudo-zero order. In the case of cholinesterase the substrate is acetylcholine and the reaction proceeds as follows:

$$(CH_3)_3NCH_2CH_2O_2CCH_3 + H_2O \xrightarrow{ChE} (CH_3)_3NCH_2CH_2OH + CH_3CO_2H$$

The activity of tissue preparations containing cholinesterase may be determined in two general ways. The rate of consumption of substrate may be measured colorimetrically (*5*) or the rate of production of acetic acid may be measured by allowing the reaction to proceed in the presence of a bicarbonate buffer in a Warburg apparatus. Carbon dioxide is given off as the acid appears and the rate can be measured manometrically by measuring the rate of change in pH in a specially buffered reaction mixture (*7*), or by measuring the rate at which alkali must be added to an unbuffered reaction mixture to maintain constant pH (*9*). The last method may be done manually or automatically.

The method described here is similar to the last method, except that constant pH is maintained by removing hydrogen ions electrolytically at the same rate as they are formed by the enzyme reaction. This is accomplished automatically by a feed-back system somewhat resembling that described by Lingane (*6*).

INSTRUMENTATION

A schematic diagram of the instrument is shown in Figure 1.

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The electrolytic cell is a modified version of the one used by Epstein, Sober, and Silver (*4*). It consists of two compartments, *A* and *B*, separated by a semipermeable (parchment) membrane, *C*, and each equipped with a platinum electrode, *D*, *E*. The cathode compartment, *A*, is further equipped with a stirrer (magnetic or Vibro-Mix), *F*, pH electrodes *G*, gas inlet *H*, and thermometer if desired. Compartment *A* is sealed and a slight positive pressure of nitrogen is maintained over it to prevent diffusion of carbon dioxide into the cell. Its size may be varied according to the needs of the reaction being studied; 15-, 50-, and 75-ml. capacities have been used in this laboratory.

The type of platinum electrode used in the cathode compartment is critical. Beckman platinum electrodes (No. 281) have been found satisfactory, but ordinary platinum wire may be unsatisfactory unless the exposed surface is kept very small. If a large surface of platinum is exposed, the electrolysis may appear to be much less than 100% efficient, and there may be marked pH drift when the electrolysis is terminated. No explanation for these phenomena has been found.

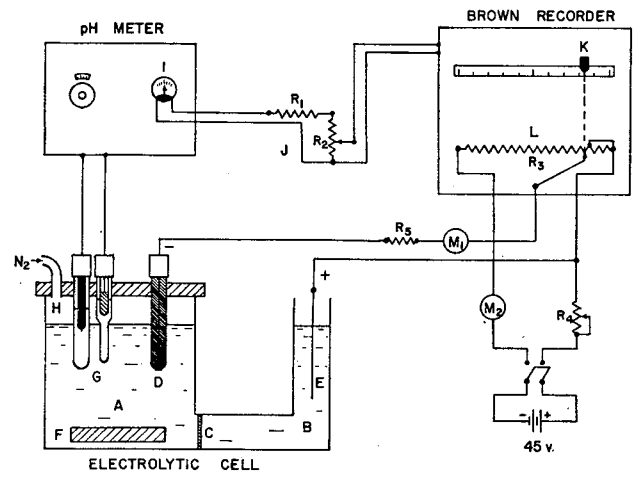

Figure 1. Schematic diagram of automatic electrolytic titrator

R_1. 10,000 ohms, 0.5 watt
R_2. Helipot, 10-turn, 1000 ohms (Beckman)
R_3. Potentiometer, 10,000 ohms tapped at 9000 ohms (General Radio)
R_4. 20,000 ohms, 5 watts
R_5. 500 ohms, 0.5 watt
M_1. Multirange milliammeter, 0–1, 0–5, 0–10, 0–25, 0–50 ma.
M_2. Milliammeter, 50 ma.

The pH electrodes are used in the usual manner with a Beckman pH meter (Model G), somewhat modified. Its design requires that a definite current pass through the null meter, *I*, in order to bring it to the zero or balanced position. The voltage drop existing across the meter is utilized to activate a Brown-Honeywell strip-chart recorder in such a way that the recorder follows the movements of the null meter needle. The two units are coupled through a voltage divider, *J*, which permits adjustment of the position of the recorder pen relative to the magnitude of the current passing through the null meter.

Some calomel reference electrodes are sensitive to a pressure differential between the inside and outside of the electrodes. This trouble was eliminated by connecting the electrode opening to the nitrogen inlet.

The feed-back system consists of a potentiometer, *L*, coupled

mechanically to the pen drive of the recorder in such a way that an increasing voltage is applied to the electrolytic cell as the pen, K, moves to the left.

OPERATION

In general, the instrument is operated by placing a suitable electrolyte containing an enzyme sample and its substrate in the cathode compartment, A, and the electrolyte only in the anode compartment, B. The pH meter and recorder are activated and the battery circuit is closed. The instrument then equilibrates in such a way as to keep the pH constant. When the pH is constant, the rate of hydrogen ion production due to the enzymatic reaction may be equated to the rate of removal of hydrogen ions by electrolysis, which in turn may be calculated by applying Faraday's law to the equilibrium current. Thus, multiplying the equilibrium current in milliamperes by the factor 0.621 μeq. per ma.-minute gives the reaction rate in microequivalents per minute.

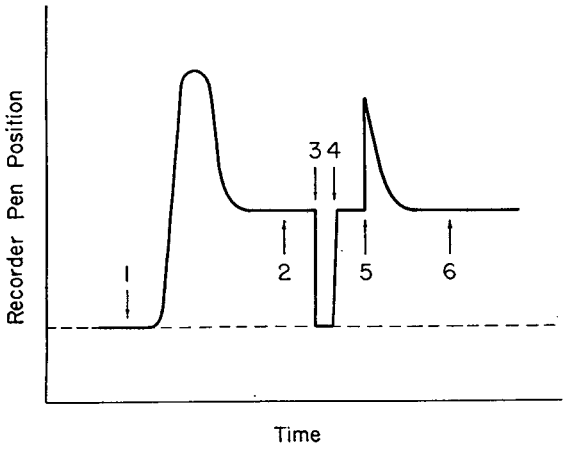

Figure 2. Typical curve obtained during process of making measurement

The way in which automatic control of pH is achieved may be seen from the schematic diagram (Figure 1).

If no acid is being produced in compartment A and the pH in the compartment is the same as that set on the pH meter, the null meter will be steady at the zero mark. If voltage divider J is adjusted so that the recorder pen and potentiometer L are at the electrical zero of the potentiometer, the battery switch may be closed without causing a change in the cell.

If, now, acid is produced at a constant rate in A, the decrease in pH will be reflected in the leftward movement of the null meter needle, the recorder pen, and the potentiometer contact. This causes a voltage to appear at the platinum electrodes, resulting in electrolytic removal of hydrogen ions from the cathode compartment. The pH will continue to decrease and the voltage to increase until the removal of hydrogen ions is just as fast as the production of hydrogen ions. At this point the pH will remain constant and a dynamic equilibrium will have been reached.

In practice it may be necessary to measure the rate of a process at a precisely known pH. The equilibrium described above occurs at a pH lower than that set at the pH meter. In order to equilibrate at the set pH the instrument must be manipulated as follows (see Figure 2):

After the generation of acid has been started, 1, and the instrument has reached its preliminary equilibrium, 2, the glass electrode is temporarily disconnected by releasing the push button on the pH dial. This brings the pointer of null meter I and consequently the position of the recorder pen back to zero, 3. Immediately, potentiometer J is adjusted, 4, to bring the pen to its preliminary equilibrium position. The push button is then depressed, 5, and the instrument allowed to equilibrate, 6.

If, after a determination is completed at a given pH, another is desired at a different pH, the new determination may be obtained with the same sample by setting the drum of the pH meter to the desired value and following the readjustment procedure outlined above. The time to reach each equilibrium ranges from 0.5 to 3 minutes with cell volume of 13 ml. The pH during a determination is maintained accurately to within 0.01 pH unit.

CALIBRATION

The instrument was calibrated by adding standardized hydrochloric acid to the cathode compartment at measured rates. The current necessary to keep the pH constant was recorded and compared with the rate of acid addition. In a series of five runs using electrodes with small surface areas, the error ranged from 0.2 to 2.8%, the electrolytic value being too high in all cases. Some trouble was encountered in this type of calibration due to fluctuations in the pH meter caused by inhomogeneous concentration in the cell. These troubles do not occur in studying reactions in which hydrogen ions are produced throughout the solution.

That the cholinesterase activity measured by the instrument is proportional to the concentration of enzyme in the cell was shown by varying the amount of enzyme source (blood) added to the cell and plotting it against the observed equilibrium current (see Figure 3). The media used in the cell for these determinations included 0.1M to 2.0M solutions of sodium chloride, sodium bromide, potassium chloride, and potassium bromide, with and without added 0.01M magnesium chloride. Sample volumes up to 2.0 ml. were used in a cell containing 68 ml. All measurements were made at pH 7.3, with a substrate concentration of 0.004M acetylcholine chloride (Merck). All the graphs of concentration *vs.* activity obtained under these limited conditions were linear. The slopes of the graphs varied with the specific activity of the enzyme.

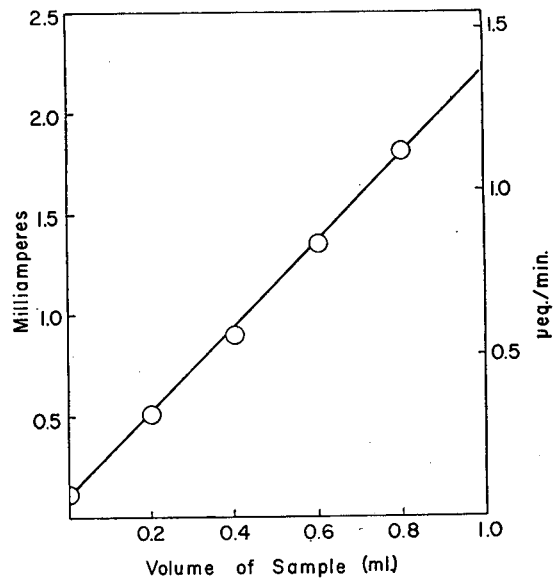

Figure 3. Relationship between enzyme sample volume and apparent activity of dog whole blood

In 0.5M NaCl, 0.01M $MgCl_2$, 0.004M AcCh solution at pH 7.3

The intercept of the graph at zero sample volume corresponds to the rate at which acetylcholine chloride is split in the absence of cholinesterase in a solution of pH 7.3. In routine determinations of enzyme activity this value is considered as a blank and is subtracted from the observed equilibrium current. It is reproducible and depends upon the concentration of acetylcholine chloride and upon the total amount of solution in the cell.

The precision of the instrument has been determined by measuring the cholinesterase activity of 10 aliquot portions of a preparation of goat red blood cells. The standard deviation was found to be ±0.04 μeq. per minute.

DISCUSSION

Thus far, the instrument has been used only in studying the enzyme system cholinesterase. The enzyme activity of the blood of humans, dogs, rats, goats, and rabbits, and of rabbit brain has been studied. The instrument's operation has been equally satisfactory with these different tissues and it should be satisfactory with homogenates of other tissues.

A particularly useful application of the instrument in the field of enzymes is the study of enzymatic activity as a function of pH, temperature, or ionic strength. The procedure for varying pH was described above. Its advantages with respect to time and sample economy are obvious. Temperature could be varied systematically if the electrolytic cell were thermostated, and the change of enzyme activity with temperature could easily be determined. Ionic strength could be varied by adding calculated amounts of salts to the cell after each equilibration.

Although the instrument has been designed to measure pseudo-zero–order reaction velocities, it could be used to measure first-order reaction velocities. Rate constants could be evaluated in the usual way, except that the milliammeter readings would be substituted for concentrations of reactive species as follows:

$$\log \frac{C_0 - x_1}{C_0 - x_2} = \log \frac{v_1}{v_2} = \log \frac{I_1}{I_2} = \frac{k(t_2 - t_1)}{2.303}$$

where

C_0 = initial concentration of reactant
x_1 and x_2 = amounts reacted at time t_1 and t_2, respectively
v_1 and v_2 = reaction velocities at time t_1 and t_2, respectively
I_1 and I_2 = current readings at time t_1 and t_2, respectively
k = specific rate constant

In this type of determination the pH must necessarily increase. However, the increase may be made so small that it will not appreciably affect the reaction nor the observed current.

Dynamic processes other than chemical reactions, such as gaseous, ionic, or liquid diffusion or solution rates, could be studied in this instrument after suitable modifications of the electrolytic cell, the sensing element, and the electrodes.

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Ultraviolet Spectrophotometric Determination of Phosgene with Aniline

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An ultraviolet spectrophotometric method has been developed for the determination of phosgene based upon the absorption of the 1,3-diphenylurea formed when phosgene is allowed to react with aniline in aqueous solution. The method is sensitive, specific, and capable of good precision.

THE aniline method for the determination of phosgene was first described by Kling and Schmutz (*5*) and shortly afterwards was successfully used by Biesalski (*1*) in studying the thermal decomposition of carbon tetrachloride. The method consisted in bubbling the gas to be tested through water saturated with aniline and weighing the 1,3-diphenylurea (carbanilide) formed. Olsen and coworkers (*7*) studied this method and found that 100 ml. of the aqueous aniline solution dissolves about 5.5 mg. of 1,3-diphenylurea. They therefore modified the procedure by first saturating the solution with diphenylurea to eliminate solubility errors. Comparing this technique with the sodium hydroxide (*2*), silver nitrate (*7*), and sodium iodide–acetone methods (*4*), these investigators recommended their procedure and that involving the use of sodium iodide and acetone.

General acceptance of the aniline method by industry testifies to its practicality. However, it has some inherent weaknesses. Very small precipitates are difficult to handle. Some foreign materials may precipitate, giving high results. The solubility of diphenylurea may vary with the conditions under which the sample is taken. On consideration of these variables it seemed of interest to develop a new method which would be more sensitive and more specific.

The variation in the absorption spectrum of aniline with pH has been studied by Tischler and Howard (*9*) from 305 to 255 mμ. In acid solution the absorbance is much smaller than in basic solution and the spectrum is similar to that of benzene (*3*, *6*). The ultraviolet absorption spectrum of 1,3-diphenylurea in ethanol has been reported by Schroeder and coworkers (*8*). The spectra of these compounds in methanol were studied in the present investigation (Figures 1 and 2). It was found that fairly small quantities of 1,3-diphenylurea can be determined in the presence of relatively large amounts of aniline in acidic methanol. Although both compounds exhibit absorption maxima at 254.5 mμ, the absorbance of 1,3-diphenylurea on a weight basis is 93.6 times as intense as that of aniline. Aniline also exhibits a sharp absorption peak at 260.5 mμ, which can conveniently be used to determine the quantity remaining after the phosgene has reacted.

APPARATUS AND REAGENTS

A Cary recording spectrophotometer, Model 11MS, with matched 1-cm. silica cells, was used for absorbance measurements. The slit control was set to produce a slit width of 0.12 mm. at 254.5 mμ. A manually operated spectrophotometer may be used, if enough points are plotted.

The spectrum of 1,3-diphenylurea was determined on solutions of a recrystallized product, melting point 235.5–7° C. Freshly distilled aniline was used to prepare solutions in water, in such concentrations that 50 ml. contained about 2 mg. per mg. of

phosgene anticipated, plus an excess of 50 mg. Phosgene (99.5 mole %) was obtained from The Matheson Co. All other reagents were of analytical quality.

CALIBRATION

Dissolve approximately 0.5 gram of 1,3-diphenylurea (weighed accurately to the nearest milligram) in methanol. Add 3 ml. of 36% hydrochloric acid and dilute to 100 ml. Take aliquots and make dilutions in such a way that the final dilution contains about 0.5 mg. per 100 ml. of methanol. Scan the spectrum of a portion of this solution in the ultraviolet from 300 to 210 mμ, using acidic methanol as a reference solution. Read the absorbance at 254.5 mμ and divide the diphenylurea concentration by the absorbance to calculate a coefficient, C. This should have a value of about 0.615 mg. per 100 ml. per absorbance unit when a 1-cm. light path is used.

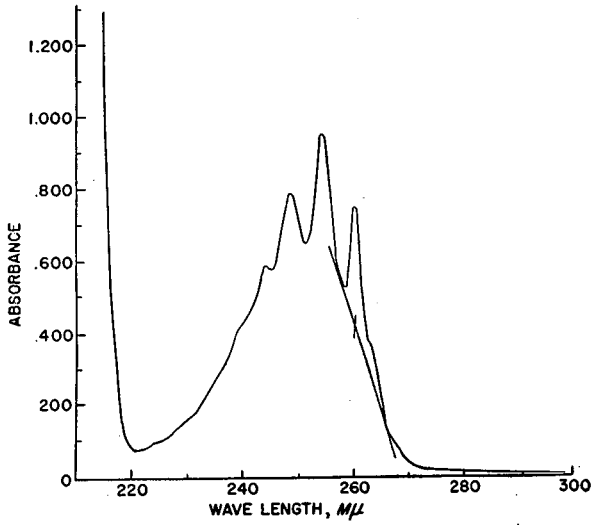

Figure 1. Ultraviolet absorption of aniline hydrochloride in methanol

55.9 mg. per 100 ml.
1.00-cm. cells

In a similar way prepare a solution of 50 mg. of aniline (weighed accurately to the nearest 0.1 mg.) in 100 ml. of methanol to which 1 ml. of hydrochloric acid has been added. Scan the spectrum from 300 to 210 mμ. Draw a base line through the minimum located at 258 mμ and tangent to the curve at about 267 mμ (see Figure 1). Read the absorbance at 260.5 mμ and subtract the base-line absorbance at the same wave length to obtain a net absorbance. Read the absorbance at 254.5 mμ and determine the ratio, R, of the total absorbance at 254.5 mμ to the net absorbance at 260.5 mμ. This should have a value of about 2.80.

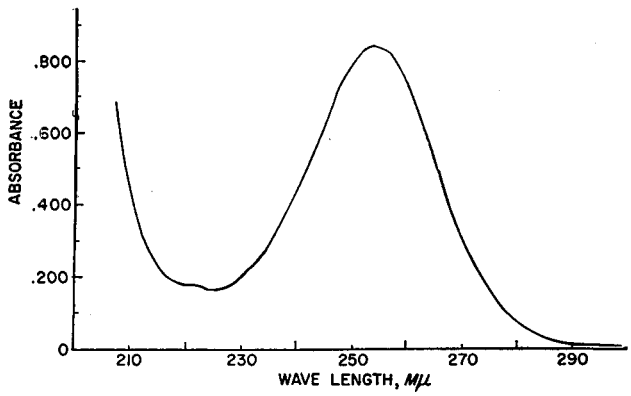

Figure 2. Ultraviolet absorption of 1,3-diphenylurea in methanol

0.517 mg. per 100 ml.
1.00-cm. cells

Table I. Determination of Phosgene

Phosgene Taken, Mg.	Phosgene Found, Mg. 1st bubbler	2nd bubbler	3rd bubbler	Total	Recovery, % 1st bubbler	Total
3.95	3.77	0.10[a]	0.04	3.91	95.4	99.0
3.97	3.87	0.02	0.02	3.91	97.5	98.5
6.26	5.96	0.07[a]	0.02	6.05	95.2	96.7
6.85	6.78	0.04	0.04	6.86	99.0	100.1
7.47	7.46	0.05	0.04	7.55	99.8	101.0
7.83	7.53	0.03	0.02	7.58	96.3	96.9

[a] Results high because gas was passed through bubblers too rapidly.

PROCEDURE

Bubble the gas under investigation through 50 ml. of the aqueous aniline solution (see Apparatus and Reagents) at a rate such that not more than 2 mg. of phosgene is passed per minute. Transfer the solution and any precipitate which may have formed into a 250-ml. volumetric flask with methanol. Add 2 ml. of concentrated hydrochloric acid. Dilute with methanol. Take an appropriate aliquot and dilute it to 100 ml. with methanol. Scan the spectrum of a portion of this solution in a 1-cm. absorption cell from 300 to 240 mμ. Determine the net absorbance at 260.5 mμ and multiply by the absorbance ratio, R. Subtract the result from the absorbance at 254.5 mμ to obtain the absorbance due to 1,3-diphenylurea. Call this quantity A.

Milligrams of phosgene in 100 ml. of solution = $A \times C \times 0.466$. The factor 0.466 represents the molecular weight ratio of phosgene to diphenylurea.

RESULTS AND DISCUSSION

In order to test the method, small quantities of phosgene were weighed in sealed capillary tubes. These tubes were broken at the stopcock in the apparatus shown in Figure 3 and the phosgene was bubbled slowly through three gas-absorption bubblers, each containing 50 ml. of the aniline solution. The results are tabulated in Table I.

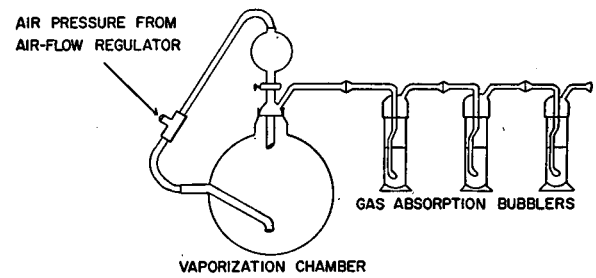

Figure 3. Apparatus for determination of phosgene

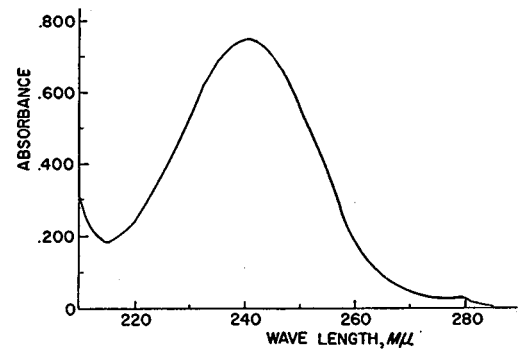

Figure 4. Ultraviolet absorption of acetanilide in methanol

0.70 mg. per 100 ml.
1.00-cm. cells

Although the results are not always quantitative, they all show better than 95% recovery in the first bubbler. In the runs

Table II. Comparison of Ultraviolet and Gravimetric Methods for Stack Gas Analysis

Sample No.	Weight of Ppt., Mg., Gravimetric Method	1,3-Diphenylurea in Ppt., Mg., Ultraviolet Method
1	54.3	14.0
2	9.6	8.1

in which the gas flow was slowed down to rates such as those encountered in practice, the recoveries are above 97.5%. This is considerably better than could be expected from the gravimetric method.

Acetyl chloride is very easily hydrolyzed and in the presence of water only a small amount reacts with aniline to form acetanilide. This compound shows an absorption maximum at 240 mμ in methanol (Figure 4). Chloroacetyl chloride quantitatively forms α-chloroacetanilide in aqueous aniline. It also shows an absorption maximum at 240 mμ in methanol.

APPLICATION TO STACK GAS ANALYSIS

The precipitation of materials other than 1,3-diphenylurea may cause high results by the gravimetric procedure. This was emphasized by the analysis of two samples from a titanium chlorinator stack. The phosgene was precipitated as 1,3-diphenylurea according to the procedure of Olsen and coworkers (7) and the precipitate was weighed. The precipitate was then dissolved in methanol and the 1,3-diphenylurea was determined by the ultraviolet method. The results are shown in Table II. As diphenylurea in the precipitate must inevitably be revealed by its absorbance, it is apparent that the gravimetric method was giving high results on these samples, particularly in the first case.

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Determination of Water Content of White Fuming Nitric Acid Utilizing Karl Fischer Reagent

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A method which utilizes Karl Fischer reagent has been developed for the direct determination of the water content of white fuming nitric acid. The weighed acid sample is first neutralized by the use of pyridine-dimethylformamide solution to prevent reaction with the reagent. An excess of Fischer reagent is then added and a standard methanol-water solution is used for the back-titration. The method has shown a relative accuracy within 1% between the calculated and experimentally determined values in the absence of dissolved metallic salts. Approximately 5% variation is found in the presence of the solids. Nitrogen dioxide concentrations of less than 1.5% do not interfere with the determination.

A DIRECT method for the determination of the water content of white fuming nitric acid, using Karl Fischer reagent, was developed in this laboratory in 1950. Mitchell and Smith (2) had reported the use of this method for the determination of water in concentrated nitric or sulfuric acids, but the method had not been extended to the white fuming nitric acid system. Later, Eberius (1) used the Karl Fischer reagent for the determination of water in mixed acid. The following procedures were developed for the determination of the water content of white fuming nitric acid containing small quantities of nitrogen dioxide and dissolved metal salts.

APPARATUS AND REAGENTS

Apparatus. Covered tall-form Berzelius beaker with openings for two burets, stirrer, and end-point indicator.

Dead-stop end-point indicator (3).

Reagents. All reagents were chemically pure or better.

White fuming nitric acid was prepared by reaction of concentrated sulfuric acid and sodium nitrate. Distillation was conducted under moderate vacuum, and dry nitrogen was passed through the distilled product to remove nitrogen oxides. Acids of 0.08 to 0.6% water content were made by this method; in the presence of phosphorus pentoxide, acids of so-called negative water contents have been prepared which contain small quantities of nitrogen pentoxide.

Karl Fischer reagent. To prepare 2 liters of Karl Fischer reagent, use 538 ml. of pyridine, 169.4 grams of iodine, 1334 ml. of absolute methanol, and 90 ml. (128 grams) of sulfur dioxide. Any of the materials which contain over 0.1% water by weight should be dried and distilled before using.

Methanol-water solution, prepared by adding weighed quantities of water to anhydrous methanol.

Pyridine-dimethylformamide solution, 2 to 1 by volume.

Standard sodium hydroxide solution, checked with standard hydrochloric acid solution standardized against sodium acid phthalate.

Standard ceric sulfate solution, prepared from ceric ammonium sulfate and standardized against arsenous oxide.

Standard ferrous sulfate solution, prepared from ferrous ammonium sulfate and standardized against ceric sulfate solution.

Liquid nitrogen dioxide, 98.0%, Matheson Co., Inc.

Ferric nitrate, $Fe(NO_3)_3 \cdot 9H_2O$.

Chromic acid.

Nickel nitrate, $Ni(NO_3)_2 \cdot 6H_2O$.

Aluminum nitrate solution, prepared by dissolving aluminum (99% pure) in white fuming nitric acid of known composition.

PROCEDURE

The classical method for the analysis of the distilled white fuming nitric acid was utilized. Nitric acid was determined by direct titration with standard base, nitrogen dioxide by reaction with excess ceric sulfate and back-titration with ferrous sulfate

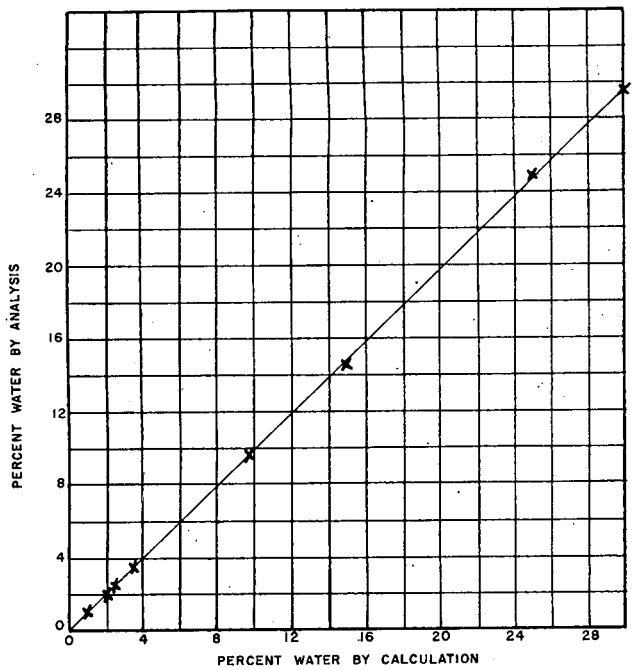

Figure 1. Comparison of calculated water to water found in white fuming nitric acid by Karl Fischer method

Table I. Water Content of White Fuming Nitric Acid

(Comparison of calculated water content with that found by Karl Fischer method)

% Water by Calculation	% Water by Analysis
	0.56
1.07	1.05
2.01	2.00
2.30	2.28
3.28	3.31
9.63	9.76
14.40	14.76
25.06	25.16
29.55	29.87

solution, and water by difference. Weighed quantities of water were added to the frozen acid to prepare the more dilute solutions. Acids of higher nitrogen dioxide content were prepared by adding nitrogen dioxide to the white fuming nitric acid. The compositions of these prepared acids were computed from the available weight relationships.

The water equivalence of Karl Fischer reagent was obtained by permitting weighed quantities of water to react with excess Karl Fischer reagent and back-titrating with methanol-water solution, using a dead-stop end-point indicator. The ratio between Karl Fischer reagent and methanol-water solution was established daily. The white fuming nitric acid solutions (1 to 2 grams) were neutralized with excess pyridine-dimethylformamide solution (10 ml.) to prevent interference with Karl Fischer reagent. Caution must be used in adding the acid to the base to avoid violent reaction. A slight excess of Fischer reagent is added to the neutralized acid solution in the water analysis, and the excess is back-titrated with methanol-water solution to the dead-stop end point. Blank determinations on all reagents used in the analysis are performed, and the results are corrected accordingly.

RESULTS AND DISCUSSION

Because Mitchell and Smith had shown that the Karl Fischer method was accurate for the determination of water in solutions containing 70% nitric acid, it was believed that the accuracy of the determination in more concentrated solutions (where available techniques were not completely accurate) could be established by proving a straight-line function. Accordingly, eight acid solutions of known water content were prepared by the dilution technique described above and analyzed by the Karl Fischer method. Results are shown in Table I and Figure 1. Statistical analysis of the data, using Youden's procedures (*4*), gives a standard deviation of the intercept equal to 0.0975 and a t value for the intercept (7 degrees of freedom) of 1.200. The 5% critical value for t (7 degrees of freedom) is 2.365; therefore, there is insufficient evidence to maintain that the intercept differs from zero by more than can be attributed to the analytical errors. Likewise, the standard deviation of the slope (8 degrees of freedom) is 1.744. The value of t obtained from this computation is less than the critical value of 2.306 (5% probability level), so that the variation of the slope from unity is not considered significant. These results indicate that no blank is required in the water titration, and that the Karl Fischer method can be used for accurate analysis of water in white fuming nitric acid at all concentration levels.

To ascertain the effect of nitrogen dioxide upon the titration, liquid nitrogen dioxide was weighed into samples of acid of known water content, and the samples were analyzed for water by the Karl Fischer procedure. Results are presented in Table II. It is apparent that for concentrations of nitrogen dioxide below 1.5% no interference occurs. Unconfirmed data indicate that this method is applicable up to nitrogen dioxide concentrations of 2.5 to 3.0%. Above this concentration low results for water are obtained.

Table II. Determination of Water in White Fuming Nitric Acid Containing Various Concentrations of Nitrogen Dioxide

NO_2 Added, Wt. %	Water, Wt. % Calcd.	Found
0.50	0.51	0.51
1.54	0.98	0.97

The effect on the Fischer method of metallic salts commonly found dissolved in commercial white fuming nitric acid was investigated by the addition of these compounds to acid samples of predetermined water content. Water analysis for these solutions are recorded in Table III. Although differences between calculated and experimental values are higher than desired, the results are not unreasonable in view of the hygroscopic nature of the solids. Because the quantities of solids used were several times the concentration normally encountered in commercial acid, it is concluded that these materials offer no interference in the analysis.

Table III. Determination of Water in White Fuming Nitric Acid Containing Dissolved Salts

Compound	Compound Added, Wt. %	Water, Wt. % Calcd.	Found
$Ni(NO_3)_2.6H_2O$	3.42	1.58	1.73
$Cr(NO_3)_3.9H_2O$	2.52	1.31	1.23
$Fe(NO_3)_3.9H_2O$	4.23	5.05	5.09
Mixed solids[a]	2.60	2.68	2.51
$Al(NO_3)_3$	0.106	5.15	5.01

[a] Composed of 90.35 wt.% $Fe(NO_3)_3.9H_2O$, 5.37% CrO_3, 4.34% $Ni(NO_3)_2.6H_2O$.

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Ultraviolet Spectrophotometric Determination of Zirconium

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Zirconium tetramandelate dissolves in aqueous ammonia, forming a soluble, saltlike compound that exhibits maximum absorbance at a wave length of 258 mμ. This is used as the basis of a spectrophotometric method for the determination of milligram amounts of zirconium in the presence of aluminum, iron, and titanium.

THE determination of zirconium using mandelic acid as precipitant was devised by Kumins (*2*). In this method the zirconium tetramandelate precipitate is ignited to the oxide, which is weighed. Kumins observed that zirconium tetramandelate is completely soluble in aqueous solutions of ammonia, forming a clear colorless solution. Hahn and Weber showed that a soluble, saltlike compound is formed in this reaction (*1*).

Spectrophotometric studies of solutions obtained by dissolving zirconium tetramandelate in ammonia (using 1-cm. quartz cells in a Beckman Model DU spectrophotometer) showed absorption in the ultraviolet region. Maximum absorbance occurred at a wave length of 258 mμ. The absorbance curve is given in Figure 1. The absorbance is probably caused by the phenyl groups of the zirconium tetramandelate molecule, as ammonium mandelate solutions give an identical absorbance spectrum. The possibility of using this absorbance as an analytical method for the determination of zirconium was investigated.

EXPERIMENTAL

Varying quantities of zirconyl ions were precipitated with mandelic acid and the precipitates were dissolved in about 20 ml. of 6*M* ammonia. The resulting solutions were diluted to 50 ml. with distilled water. These solutions were stable for about 2 days. After this time hydrolysis occurred and zirconium hydroxide was precipitated. The absorbance was measured at 258 mμ. Results when plotted show that Beer's law is obeyed over the entire range. These data indicate that the method may be useful for the determination of small amounts of zirconium. After experimentation the following procedure was devised.

PROCEDURE

A solution containing 0.5 to 50 mg. of zirconium is placed in a 250-ml. beaker and diluted to about 20 ml. with distilled water. Twenty milliliters of 12*M* (concentrated) hydrochloric acid are added and the resulting solution is heated to about 85° C. Twenty-five milliliters of a 1*M* (about 15%) solution of mandelic acid are added dropwise with stirring. The solution is maintained at about 85° C. for 0.5 hour. The sample is allowed to cool, then to stand for about 24 hours. The precipitate is filtered by suction using a sintered-glass crucible of medium porosity. The precipitate is washed five times with a solution containing 5% mandelic acid and 2% hydrochloric acid, three times with 95% ethyl alcohol, and twice with ethyl ether. The precipitate is treated with individual 5-ml. portions of 6*M* ammonia until it is completely dissolved. (Usually three or four treatments are necessary.) The crucible is finally washed three times with 5-ml. portions of distilled water and these washings are combined with the previous solution. The resulting solution is transferred to a 50-ml. volumetric flask and diluted to the mark with distilled water. The absorbance is measured at 258 mμ using the same quantity of 6*M* ammonia diluted to 50 ml. as reference. The amount of zirconium is determined by reference to the standard curve.

DISCUSSION

The amount of ammonia used to dissolve the zirconium tetramandelate is not critical; identical results were obtained in solutions 0.72*M* to 2.16*M* in ammonia. The alcohol and ether wash ensures complete removal of any excess mandelic acid.

The interference of diverse ions was studied by preparing samples containing a known amount of zirconium, adding known amounts of foreign ions, and analyzing the samples by the pro-

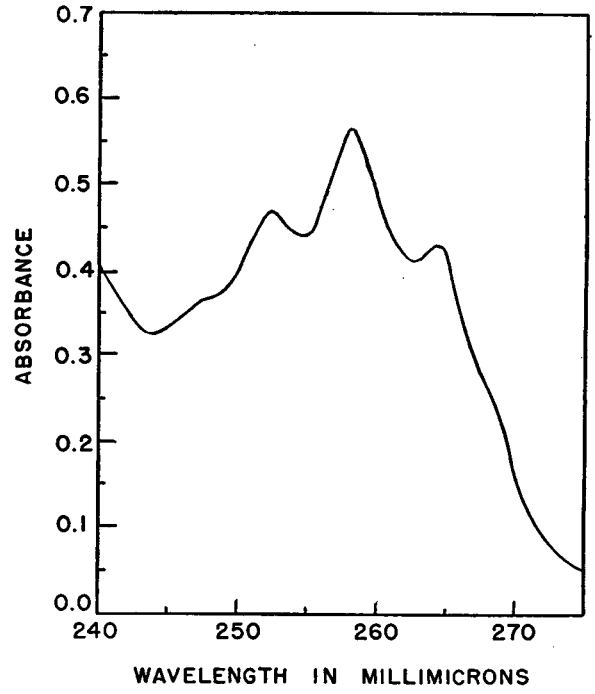

Figure 1. Ultraviolet absorption spectrum of ammonium zirconium tetramandelate

Table I. Determination of Zirconium in Presence of Aluminum, Ferric, and Titanium Chlorides

Sample	Ion Added, Mg. Al	Ti	Fe	Zirconium, Mg. Taken	Found	Error, %
1	20	20	..	0.505	0.99	+96.0
2	20	20	20	0.505	1.30	>100
3	10	10	10	1.01	1.58	+56.4
4	10	10	10	2.02	3.18	+57.4
5	10	10	10	5.05	6.29	+24.6
6	20	..	..	10.1	10.1	0
7	..	..	20	10.1	10.9	+7.9
8	..	20	..	10.1	10.3	+2.0
9	20	20	20	10.1	11.1	+9.9
10	100	..	..	10.1	10.2	+1.0
11	..	..	100	10.1	11.5	+13.9
12	..	100	..	10.1	11.1	+9.9
13	100	100	..	10.1	11.1	+9.9
14	..	100	100	10.1	11.4	+12.9
15	100	100	100	10.1	12.1	+19.8

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cedure given previously. Aluminum(III), iron(III), and titanium(IV) were selected because they are frequently associated with zirconium in ores and alloys and are the most likely to cause interference. It was observed that the nitrate ion interferes by causing incomplete precipitation of the zirconium tetramandelate. The data given in Table I indicate that small amounts of zirconium can be determined by the above method in the presence of 100 mg. of aluminum(III), 10 mg. of iron(III), and 20 mg. of titanium(IV). Errors occur when larger quantities of these ions are present. The accuracy is poor in samples containing less than 1 mg. of zirconium.

This method should prove useful for the rapid determination of small amounts of zirconium, as it requires no final ignitions and weighings.

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Oscillometric Determination of Fluoride

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A procedure is described for the determination of macro quantities of fluoride. The method employs a modification of the Willard and Winter steam-distillation procedure, followed by accurate adjustment of the pH, and subsequent titration with thorium nitrate using a high frequency oscillometer for end point detection. For the range of 3 to 8 mg. of fluoride per 100 ml. of solution, the average accuracy is within 0.2%. The method is rapid and has the particular advantage of being easily adaptable to automatic recording.

THE determination of macro quantities of fluoride is a problem of major analytical concern. Considerable work has been published on techniques of decomposition and separation, the latter commonly using a modification of the Willard and Winter steam-distillation procedure (*25*). The separated fluosilicic acid can be analyzed gravimetrically or volumetrically.

Gravimetric procedures, such as precipitation of calcium fluoride (*20*), lead chlorofluoride (*21*), lead bromofluoride (*7*), lanthanum fluoride (*14, 18*), triphenyltin fluoride (*1*), and bismuth(III) fluoride (*6*), are tedious. The precipitates are often of variable composition and have appreciable solubilities.

Volumetric methods, such as the titrations with thorium nitrate (*25*), aluminum chloride (*13*), cerous chloride (*5*), ferric chloride (*8*) or hydroxide (*17, 22*), are subject to many difficulties. In general, the indicator color changes are extremely subtle, making the method unsatisfactory for the inexperienced analyst or technician. Rickson (*19*) states that the widely used thorium nitrate titration and its modifications "appear to be based more on personal choice than on any fundamental reasoning." For these reasons, recent interest has centered around the possibility of adapting a titration procedure to a recording instrument.

It was the purpose of this investigation to determine the applicability of a high frequency oscillometer to one of the fluoride reactions. High frequency automatic titrators are adaptable to a variety of reactions including precipitation, acid-base, complex formation, a few oxidation-reduction reactions, and reactions in organic solvents; therefore, they seemed to have promise. Good end point detection has been found by this method for such inorganic ions as thorium (*4*), chloride (*3*), beryllium (*2*), calcium (*12*), magnesium (*12*), and sulfate (*16*). Blaedel and Malmstadt (*4*) have stated that the reaction of fluoride with thorium is definitely not useful for high frequency titrations because of excess curvature in the instrument response curve in the region of the equivalence point. This curvature was not eliminated by varying conditions such as acidity and thorium concentration. Harley and Revinson (*10*) reported encouraging results in the titration of micro quantities of fluoride with thorium, using a high frequency oscillator to detect the end point. The analytical results, however, have never been published.

The advantages of the high frequency method are not due to increased accuracy or sensitivity over conventional electrometric procedures, but lie in the convenience of operation, the ability to show the end points for reactions which are masked when other indicators are used, and the absence of physical contact of electrodes with the solution. This eliminates the influence of electrode potentials as well as the possibility of electrode contamination and electrolytic alteration of the concentration. An additional advantage is the ease with which high frequency titrators may be adapted for use with automatic recorders.

A disadvantage is the uncertain relationship between the indicated end point for a reaction and the stoichiometric equivalence point.

APPARATUS AND REAGENTS

The distillation apparatus is that described by Huckabay, Welch, and Metler (*11*). A specially constructed Glas-Col heater was used to heat the distillation chamber.

The high frequency measurements were made with a Sargent Model V chemical oscillometer, operating at a frequency of approximately 5 megacycles (*24*). The instrument operates on a capacitive retune principle. The sample cell is in parallel with calibrated capacitors, and because capacitance in parallel is additive, it is necessary simply to adjust these capacitors to bring the instrument back into resonance after the addition of each titration increment. Readings are made in scale units having the dimensions of capacitance.

Two types of cells were used during the course of the work. Because of the possibility of corrosion, tests were made on a polyethylene cell similar to the glass cell of Hall and Gibson (*9*), and employed in conjunction with the Sargent oscillometric cell compensator (*24*). Satisfactory operation was obtained but the stability and sensitivity were not so good as with the 150-ml. glass titration cell supplied with the unit. The major portion of the work, including all analytical results reported here, was performed in the glass cell, which showed only minor corrosion over a 2-year period.

Solutions were stirred continuously with a motor stirrer during the titrations. Timed increments of titrant were added from a 10-ml. microburet. All glassware was calibrated.

A Beckman Model H-2 pH meter, with glass and saturated calomel electrodes, was used for the pH measurements.

Sodium fluoride, analytical reagent grade, was used as the standard. From a consideration of the stated analysis, the fluoride content was calculated to be equivalent to that of a sodium fluoride 100.0% pure. Consequently, no further purification was attempted. A solution containing 1 mg. of fluoride per milliliter was prepared by dissolving 2.2100 grams of the material, dried overnight at 140° C., to make 1 liter of solution, which was stored in a polyethylene bottle.

Table I. Analysis of Aqueous Sodium Fluoride

Fluoride Present, Mg.	Thorium Nitrate Required, Ml.	Deviation from Average, Ml.	Deviation from Average, %	Ml. Titrant per Mg. Fluoride
2.00	1.94	+0.01	0.5	0.970
2.00	1.94	+0.01	0.5	0.970
2.00	1.95	+0.02	1.0	0.975
2.00	1.90	−0.03	1.5	0.950
	Av. 1.93			0.966
3.00	2.93	0.00	0.0	0.977
3.00	2.95	+0.02	0.7	0.983
3.00	2.90	−0.03	1.0	0.967
3.00	2.92	−0.01	0.3	0.973
	Av. 2.93			0.975
4.00	3.92	+0 02	0.5	0.980
4.00	3.89	−0.01	0.3	0.973
4.00	3.91	+0.01	0.3	0.978
4.00	3.89	−0.01	0.3	0.973
	Av. 3.90			0.976
5.00	4.88	0.00	0.0	0.976
5.00	4.89	+0.01	0.2	0.978
5.00	4.87	−0.01	0.2	0.974
5.00	4.88	0.00	0.0	0.976
	Av. 4.88			0.976
6.00	5.82	−0.02	0.3	0.970
6.00	5.87	+0.03	0.5	0.978
6.00	5.84	0.00	0.0	0.973
6.00	5.83	−0.01	0.2	0.972
	Av. 5.84		0.4	0.973

Approximately 0.072*N* thorium nitrate was prepared by dissolving 10 grams of the reagent grade tetrahydrate to make 1 liter of solution.

The other materials used were of reagent quality.

PRELIMINARY EXPERIMENTS

Precipitation of lead bromofluoride was attempted in the polyethylene cell, using samples of sodium fluoride containing approximately 0.1 gram of fluoride in 80 ml. of solution. Although a break occurred in the titration curve, it did not give promise of good end point detection.

The remaining tests were made on samples containing 5 mg. of fluoride in 100 ml. of solution. Titrations were carried out by adding the titrant in 0.25-ml. increments until well past the end point, and recording the instrument reading after each addition. Instrument readings were plotted against volume of titrant added, and the end point was located from the intersection of the extrapolated straight-line portions.

The titration of fluoride with ferric chloride, involving the formation of complexes, was tried. Instrument response curves indicated that this reaction would not provide good end point detection, at least in this concentration region.

Plots of the data for the precipitation of calcium fluoride by means of calcium chloride resulted in smooth curves. The precipitation of bismuth(III) fluoride was similarly unsatisfactory. In both cases, the difficulty was probably due to the solubilities of the precipitates. The precipitation of cerous fluoride by cerous chloride was attempted in water solution and also in 50% (by volume) methanol-water mixtures. The results were better than those for the previously mentioned precipitation reactions, but did not show so much promise as the reaction with thorium nitrate, which was chosen for study.

Instrument response curves were determined for the thorium nitrate titration in water, water-ethanol mixtures, and water-dioxane mixtures. As the percentage of ethanol was increased, the change of slope in the instrument response curve at the end point became more pronounced. This, however, was accompanied by an increasing amount of curvature of the initial slope of the instrument response curve, which made it difficult to locate the end point accurately. No sharp break occurred when dioxane-water mixtures were employed. Water was selected as the best of the media studied.

Samples containing 2, 3, 4, 5, and 6 mg. of fluoride in 100 ml. of solution were titrated to establish the precision and the concentration range practical to handle. The data are presented in Table I. The greatest precision was obtained in the region of 4 to 6 mg., although results were satisfactory with only 2 mg. of fluoride present. The upper concentration limit was determined by the cell characteristics, and is considerably above 6 mg. This region was not studied, however, because too many points were required for the concentration of titrant used, making the time of titration excessive.

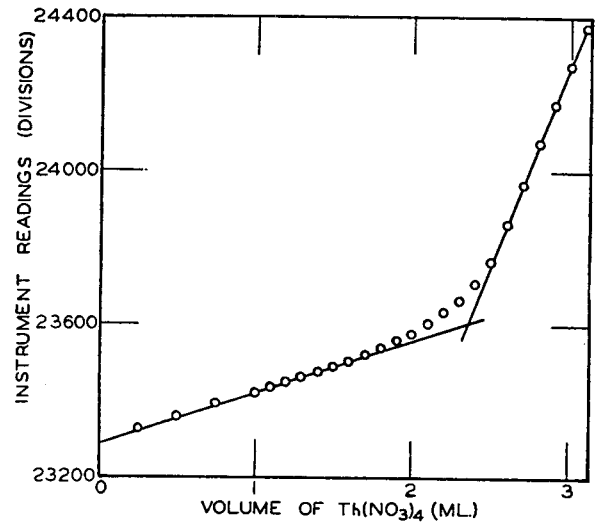

Figure 1. Typical instrument response curve for thorium nitrate titration of fluoride

The possibility of deterioration of the thorium nitrate was also considered. Milkey (*15*) has shown that thorium solutions of pH 3.0 or less do not decrease in concentration on standing. The pH of the thorium solution used in this work was 2.65; consequently, it should be stable without any addition of acid. Such a solution was used over a period of several months to titrate samples containing 5 mg. of fluoride in 100 ml. of solution. There was no significant change in titer for at least 3 months.

A thorium solution was also prepared, with nitric acid added, to see if the stability could be further increased. However, the addition of nitric acid in an amount sufficient to lower the pH to 2.44 resulted in some curvature in the instrument response curve, thus reducing the accuracy of the end point. Consequently, it seemed more practical to avoid the addition of any acid.

PROCEDURE

The procedure developed is based on the distillation of fluoride, collection as fluosilicic acid, and titration with thorium nitrate following a fixed titration pattern, at an initial pH of 5.90.

The preparation of a standard curve of milliliters of titrant *vs.* milligrams of fluoride is carried out by the same procedure used in the determination of unknown amounts of fluoride. Sodium fluoride of previously specified purity is used as the standard. It must be distilled, because direct standardization without distillation results in an appreciable error when the titrant is used for distilled samples.

Weigh out a sample of sodium fluoride, dried overnight at 140° C., containing approximately 25 mg. of fluoride. With a funnel having a tube extending below the side arm of the distillation apparatus, wash the sample quantitatively into the chamber, using not more than 30 ml. of distilled water. Add a small spatula full of finely ground soft glass and 10 ml. of fluoride-free, concentrated sulfuric acid. Rinse the funnel with a small amount of distilled water and replace the ground-glass stopper. Distill according to the directions of Huckabay, Welch, and Metler

(*11*). Collect 400 to 450 ml. of distillate in a 500-ml. volumetric flask containing slightly less than enough dilute sodium hydroxide to neutralize the fluosilicic acid produced. Approximately 0.45 ml. of 0.1*N* sodium hydroxide per mg. of fluoride is satisfactory. A piece of Tygon tubing may be used from the end of the condenser to the bottom of the flask, or the distillate may be allowed to drip directly from the condenser tip. Results obtained using both methods showed that no fluoride is lost when the tubing is not used, as long as the tip of the condenser is inside the neck of the flask.

Carry out duplicate titrations on aliquots containing 2, 3, and 5 mg. of fluoride, in the following manner. To an aliquot in the titration cell, add enough distilled water to make the total volume 100 ml. Adjust the pH to 5.90 ± 0.02 as indicated by a pH meter, using 0.1*N* and 0.01*N* sodium hydroxide. Rinse the electrodes and stirrer, using not more than 10 ml. of water, with 5 ml. usually being sufficient. Although this dilution results in a slight decrease in pH, depending on the amount of fluoride present, analytical results do not indicate a need for compensating for this effect. Place the cell in the holder and add titrant in 0.25-ml. increments, with stirring, every 30 seconds until within 1.3 to 1.5 ml. of the end point. Record instrument readings after each addition. Complete the titration using increments of 0.10 ml. Beyond the end point, increase the time interval to 60 seconds to allow for the longer time to reach equilibrium. Obtain 7 to 10 points beyond the end point. If desired, the time interval may be kept constant at 60 seconds throughout, a probable feature of automatic recording.

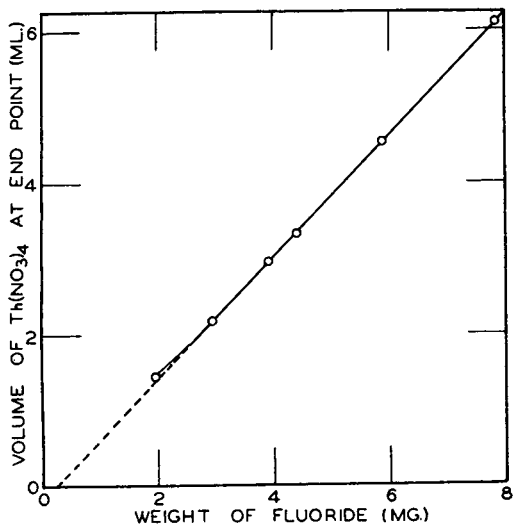

Figure 2. Standardization curve for fluoride

Plot the instrument readings *vs.* volume of thorium nitrate. The end point is located by drawing straight lines through the portion of the curve where titration increments are 0.10 ml.; the point of intersection is the end point. There is a tendency toward curvature in the initial slope; however, the end point for a given set of data is reproducible to within ±0.01 ml. A typical instrument response curve is shown in Figure 1.

Construct a standardization curve showing volume of thorium nitrate *vs.* weight of fluoride. Obtain additional points by distilling a second sample of sodium fluoride containing approximately 40 mg. of fluoride. Titrate duplicate aliquots containing 4, 6, and 8 mg. of fluoride (Figure 2). The best results are obtained when an aliquot contains a minimum of 3 mg. of fluoride. The upper limit of 8 mg. could be extended.

To analyze a sample, weigh out an amount of the dried material to contain between 15 and 50 mg. of fluoride, and distill. Establish the approximate fluoride content by a rough titration. Titrate aliquots containing the desired amount of fluoride as described in the standardization procedure; determine the fluoride content from the standardization curve.

DISCUSSION AND RESULTS

Fluorine separated by distillation from most fluorine-containing materials is usually in the form of fluosilicic acid; therefore, an attempt was made to study the precision of the titration of this acid. Samples of fluosilicic acid were neutralized with dilute sodium hydroxide, using bromocresol green indicator. These solutions were found to have pH values ranging from 5.2 to 5.7, approximating that of the sodium fluoride solutions previously used, which had a pH of 5.8. This situation was desired because no buffer was being used in the system. The results obtained from the titration of several samples, with the pH adjusted in this manner, were erratic, suggesting that the adjustment of the pH was too critical to be accomplished by an indicator. For the oscillometer cells used, the most sensitive concentration range for electrolytes lies between 0.001*M* and 0.01*M*. In order to maintain a low ion concentration, it is desirable to avoid the use of a buffer to adjust the pH. Thus, an extensive study of pH variation in the system became necessary.

Data were obtained for the change in pH of solutions containing 5 mg. of fluoride (as sodium fluoride) in 100 ml. of solution, during titration with thorium nitrate. These were adjusted to various initial pH values, and titrated. A plot of the data is shown in Figure 3.

According to Shell and Craig (*23*), the pH decreases during this titration due to the following reaction:

$$Th^{+4} + 4HF\ (weak) \longrightarrow ThF_4 + 4H^+$$

Such a decrease is observed when the initial pH is between 2.8 and 4.0 or above 5.8. Between 4.0 and 5.8, the pH is observed to increase initially and then decrease sharply. The maximum increase occurs when the initial pH is approximately 5, and is of the order of 0.3 pH unit when 5 mg. of fluoride is present, increasing in magnitude as the amount of fluoride present increases. No increase is observed for 1 mg. of fluoride. It was also noticed that when 5 mg. of fluoride was present, the first visible precipitate appeared when the mole ratio of thorium to fluoride was approximately 1 to 7. This precipitate seemed to diminish in amount beyond a mole ratio of approximately 1 to 3.5, and was no longer visible at a mole ratio of 1 to 2.5. The disappearance was independent of the pH. Solutions of fluosilicic acid were found to act the same as solutions of sodium fluoride.

As yet, no explanation for these observations has been shown to be correct. Attempts to duplicate these phenomena with titrants of calcium ion, cerous ion, and lanthanum ion failed. The maximum occurs in 50% ethyl alcohol, although it does not appear to be as pronounced. The possibility of hydrogen ion adsorption on the precipitate was considered, but it was not possible to obtain supporting evidence.

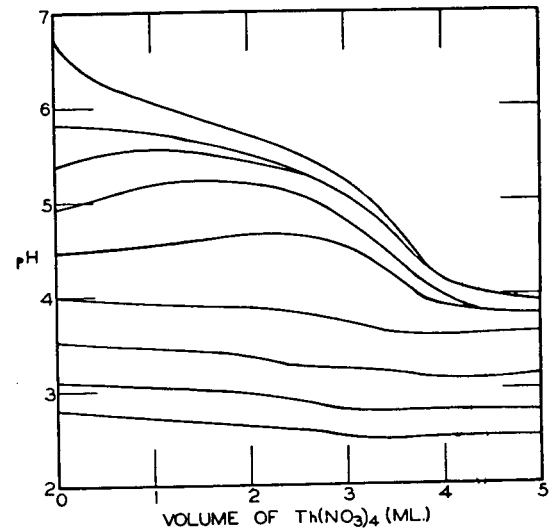

Figure 3. Variation of pH during titration of sodium fluoride with thorium nitrate at several initial pH values

The effect of initial and end point pH on the oscillometric end point was studied with the pH meter electrodes in the oscillometer cell during titration. This setup was somewhat cumbersome, owing to lack of space, but yielded the desired data. In the actual procedure the electrodes were removed during the titration. With a cell of different design, it should be possible to leave the electrode in the solution and still not have any space problem. Figure 4 is a plot of the data obtained by titrating 5 mg. of fluoride as sodium fluoride. The end point is seen to be a function of the initial pH, whereas the end point pH may

vary appreciably. The rate of change of end point is smallest for the range of initial pH values between 5.5 and 6.5. Solutions containing 5.48 mg. of fluoride as fluosilicic acid were neutralized to pH values in this range and titrated. The results showed that for initial pH values between 5.7 and 6.0, the rate of change of end point is approximately 0.01 ml. per 0.1 pH unit. This was chosen as the best analytical region.

It was also of interest to note that for the titration of 5 mg. of fluoride as sodium fluoride, the shape of the instrument response curves changed with the initial pH. This is shown in Figure 5. These results suggest that the nature of the reaction is dependent upon pH. It has not as yet been determined just what change takes place, however. If the reaction were stoichiometric with ThF_4 as the product, the theoretical equivalence point would be 3.6 ml. Figure 5 shows that the values obtained deviate significantly from this value. The inversion of the instrument response curves coincides with a minimum end point.

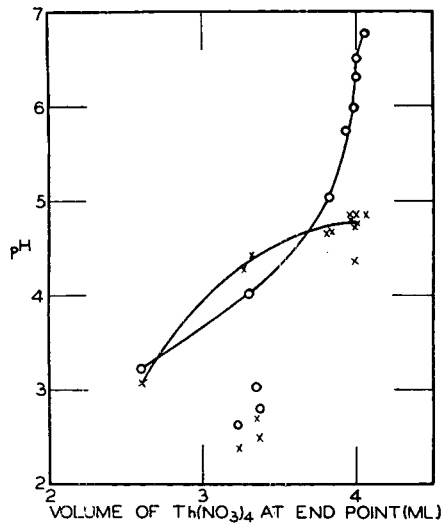

Figure 4. Effect of initial and end point pH on the oscillometric end point of thorium nitrate titration of sodium fluoride

○ Initial pH
× pH at end point

The accuracy of the method was established by analyzing samples of sodium fluoride, ammonium hexafluoroferrate(III), cadmium fluoride, and lithium fluoride. The average deviation was found to be less than 0.2%. The results are given in Table II.

ACKNOWLEDGMENT

This research was supported in part by the Atomic Energy Commission in connection with a general program of research on inorganic fluorides.

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Table II. Comparison of Results of Analyses of Sodium Fluoride, Ammonium Hexafluoroferrate(III), Cadmium Fluoride, and Lithium Fluoride

(With theoretical values)

Compound Analyzed	Mg. Titrated	Mg. Found	Fluoride Content, %	Deviation from Theoretical, %
NaF	6.33	6.38	45.6	+0.4
NaF	6.33	6.36	45.4	+0.2
NaF	3.17	3.19	45.6	+0.4
NaF	4.75	4.74	45.1	−0.1
		Av.	45.4	
		Theoretical	45.24	
$(NH_4)_3FeF_6$	3.93	3.94	51.0	+0.1
$(NH_4)_3FeF_6$	3.93	3.95	51.2	+0.3
$(NH_4)_3FeF_6$	3.93[a]	3.95	51.2	+0.3
$(NH_4)_3FeF_6$	3.93[a]	3.94	51.0	+0.1
		Av.	51.1	
		Theoretical	50.90	
CdF_2	3.44	3.44	25.3	0.0
CdF_2	3.44	3.44	25.3	0.0
CdF_2	6.87	6.85	25.2	−0.1
		Av.	25.3	
		Theoretical	25.26	
LiF	3.29	3.28	73.1	−0.1
LiF	3.29	3.28	73.1	−0.1
LiF	6.58	6.56	73.1	−0.1
		Av.	73.1	0.2
		Theoretical	73.24	

[a] Determined by analyst unfamiliar with this titration procedure.

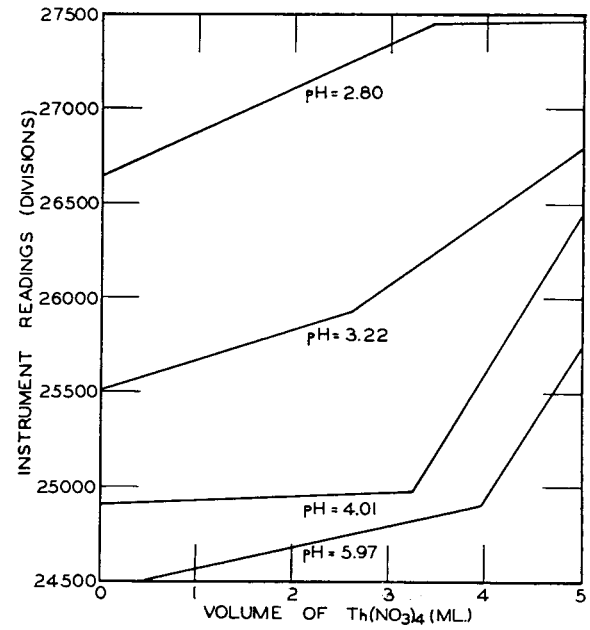

Figure 5. Effect of initial pH on shape of instrument response curves for thorium nitrate titration of fluoride

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105. Potassium Copper Dicyanide, $KCu(CN)_2$

EUGENE STARITZKY and DONALD J. WALKER[1], The University of California, Los Alamos Scientific Laboratory, Los Alamos, N. M.

Potassium copper dicyanide was prepared by evaporating at room temperature an aqueous solution containing 20 weight % potassium cyanide and 14% cuprous cyanide. Bassett and Corbet (*1*) give the stability relations of this compound in the system KCN–CuCN–H_2O at 25° C. Copper by chemical analysis was 40.8%, calculated 41.08%.

Crystal Morphology

System and Class. Monoclinic, prismatic.

Axial Elements. $a:b:c = 0.960:1:0.953$; $\beta = 102° 10'$. Groth (*2*) lists axial elements for $KCu_2(CN)_3 \cdot H_2O$ which are inconsistent with the cell dimensions determined for that compound. After interchanging a and c, Groth's figures become $a:b:c = 0.9645:1:0.9482$; $\beta = 102° 52'$. It appears likely that Rammelsberg's measurements, from which the above set of values was derived, were made on crystals of potassium copper dicyanide.

Habit. Thick tabular {100} with {001}, {110}, {$\bar{1}02$}, {011}, {111}.

Polar Angles. (100)Λ(001) = 77° 50′; (100)Λ(110) = 43° 11′. (001)Λ(011) = 42° 58′; (100)Λ(111) = 48° 23′ (calculated 48° 27′).

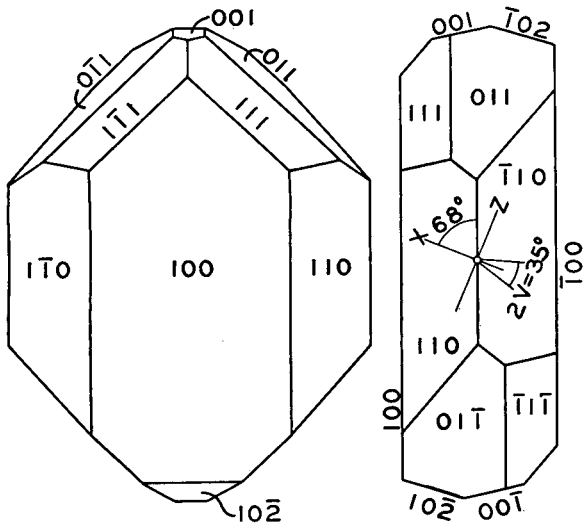

Figure 1. Orthographic projections of crystal of potassium copper dicyanide on (100) and parallel to *b*

X-Ray Diffraction Data

Space Group. $P2_1/c$ (C_{2h}^5). D. T. Cromer, of this laboratory, has determined the structure of this compound. This will be described in a forthcoming publication.

Cell Dimensions. $a_0 = 7.57$ A.; $b_0 = 7.82$ A.; $c_0 = 7.45$ A.; $\beta = 102.2°$; $a_0:b_0:c_0 = 0.968:1:0.953$; cell volume = 431 A.^{3}

Formula Weights per Cell. 4.

Formula Weight. 154.67.

Density. 2.38 grams per cc. (x-ray); 2.384 (flotation).

Optical Properties

Refractive Indices (5893 A.). $n_X = 1.589 \pm 0.002$; $n_Y = 1.705 \pm 0.003$; $n_Z = 1.718 \pm 0.005$; geometric mean 1.670.

Molecular refraction. 24.24 cc.

Partial Powder X-Ray Diffraction Pattern of $KCu(CN)_2$

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
100	7.40	7.38	65
110	5.375	5.36	50
$\bar{1}11$	4.68	4.67	20
111	4.04	4.03	25
$\bar{1}02$	3.58	3.57	25
021	3.445	3.44	50
$\bar{2}11$	3.287	3.28	100
102	3.024	3.02	70
$\bar{2}02$	2.922	2.91	25
112	2.820	2.82	65
$\bar{2}12$	2.737	2.73	10
022	2.665		
$\bar{2}21$	2.657	2.65	60
$\bar{1}22$	2.641		
		2.445	10
		2.380	5
		2.353	10
		2.268	5
		2.178	10
		2.122	40
		2.083	35
		1.973	20

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; λ(CuKα) = 1.5418 A.

[b] Relative peak intensities above background from densitometer measurements.

Optic Orientation. $Y = b$; $X \Lambda c = 68°$. Very strong inclined dispersion of the bisectrices $X_v \Lambda c > X_r \Lambda c$.

Optic Axial Angle (5893 A.). $2V_x = 35°$ with strong dispersion $r > v$.

Colorless.

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Work done under the auspices of the Atomic Energy Commission.

106. Potassium Silver Dicyanide, $KAg(CN)_2$

EUGENE STARITZKY, The University of California, Los Alamos Scientific Laboratory, Los Alamos, N. M.

Potassium silver dicyanide was prepared by evaporation at room temperature of an aqueous solution containing potassium cyanide and silver cyanide. Bassett and Corbet (*1*) give the stability relations of this compound in the system KCN-AgCN-H_2O at 25° C.

Crystal Morphology

System and Class. Trigonal, ditrigonal-pyramidal.

Axial Element. $\alpha = 67° 31'$. This is in agreement with the α derived from goniometric measurements by Fock, although this element is incorrectly given by Groth (*2*) as 66° 57′. The corresponding hexagonal axial ratio is $c:a = 2.0703$ (this figure is correctly quoted in Groth). To conform to the orientation of the x-ray cell the hexagonal morphological axes should be transformed by the matrix (210/$\bar{1}$10/002). The axial ratio after transformation is $c:a = 2.390$.

Habit. Very variable. In terms of the trigonal axes ($\alpha = 67° 31'$) various combinations of the basal pedions {111}, {$\bar{1}\bar{1}\bar{1}$} with trigonal pyramids, {100}, {$\bar{1}$00}, {1$\bar{2}\bar{2}$}, {$\bar{1}$22} and the trigonal prism {$\bar{2}$11}. Fock (*2*) and Hoard (*3*) observed pseudo-

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Partial Powder X-Ray Diffraction Pattern of Potassium Silver Dicyanide

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
00·2	8.795	8.81	25
10·0	6.408	6.35	5
10·1, 10·$\bar{1}$	6.021	6.00	40
10·2, 10·$\bar{2}$	5.179	5.17	15
00·4	4.397	4.39	30
10·3, 10·$\bar{3}$	4.326	4.30	15
11·0	3.700	3.68	5
10·4, 10·$\bar{4}$	3.626	...	..
11·2	3.410	3.40	40
20·0	3.204	...	..
20·1, 20·$\bar{1}$	3.152	3.13	100
10·5, 10·$\bar{5}$	3.084	3.07	10
20·2, 20·$\bar{2}$	3.011	3.00	15
00·6	2.932	2.92	5
11·4	2.831	2.811	60
20·3, 20·$\bar{3}$	2.812		
10·6, 10·$\bar{6}$	2.666	...	..
20·4, 20·$\bar{4}$	2.590	2.583	5
12·0	2.422	...	..
12·1, 12·$\bar{1}$	2.400	...	..
20·5, 20·$\bar{5}$	2.369	2.362	20
10·7, 10·$\bar{7}$	2.339	2.331	5
12·2, 12·$\bar{2}$	2.335		
11·6	2.298	2.291	10
12·3, 12·$\bar{3}$	2.239	2.235	5
00·8	2.199	2.191	10

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; λ(Cu$K\alpha$) = 1.5418 A.
[b] Relative peak intensities above background from densitometer measurements.

rhombohedral combinations of {100} and {$\bar{1}$00}, with or without the bases, and assumed a centrosymmetric class and space group. However, several combinations of forms without a center of symmetry have been observed with great frequency, such as {100} with {1$\bar{2}\bar{2}$}, {100} with {111} and {$\bar{1}\bar{1}\bar{1}$}, or {2$\bar{1}\bar{1}$} with the two bases.

Polar Angles. (111) Λ (100) = 67° 18′; (100) Λ (101) = 106° 2′ (106° 3′ calculated).

X-Ray Diffraction Data

Space Group. Hoard (*3*) determined the structure of this compound assuming the space group to be $P\bar{3}c$ (D_{3d}^2). As noted above, morphological development of some crystals was noncentrosymmetric, indicating the space group to be $P\bar{3}c$ (C_{3v}^4). No piezoelectric effect could be detected, however, with an instrument of the Giebe-Scheibe type.

Cell Dimensions. Within limits of experimental error cell dimensions determined were in agreement with those given by Hoard (*3*): a = 7.40 A., c = 17.59 A.; cell volume 834 A.^{3}

Formula Weights per Unit Cell. 6.

Formula Weight. 199.01.

Density. 2.38 gram per cc. (x-ray); 2.376 (flotation).

Optical Properties

Uniaxial positive.

Refractive indices (minimum deviation method).

Wave Length, Microns	n_O	n_E	n, Geom. Mean	Molecular Refraction
0.664	1.485	1.599	1.522	25.55
Na 0.5893	1.4915	1.6035	1.5279	25.79
Hg 0.5461	1.4969	1.6078	1.5330	26.00
Hg 0.4358	1.5233	1.6244	1.5563	26.93

Colorless.

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Work done under the auspices of the Atomic Energy Commission.

107. Potassium Gold Dicyanide, $KAu(CN)_2$

EUGENE STARITZKY and FINLEY H. ELLINGER, The University of California, Los Alamos Scientific Laboratory, Los Alamos, N. M.

Potassium gold dicyanide was prepared by evaporating at room temperature an aqueous solution of potassium cyanide and gold cyanide in equimolar proportion. According to Bassett and Corbet (*1*) this is the only double salt formed at 25° C. in the system KCN-AuCN-H_2O.

Crystal Morphology

System and Class. Trigonal, trigonal pyramidal.

Axial Element. α = 43° 52′.

Habit. Commonly pseudo-rhombohedral combinations of the trigonal pyramids {100} and {00$\bar{1}$}, often with one or both basal pedions {111}, {$\bar{1}\bar{1}\bar{1}$}. Noncentrosymmetric combinations of trigonal pyramids {100} and {1$\bar{2}\bar{2}$} were also observed frequently. No piezoelectric effect was observed.

Polar Angles. (100) Λ (010) = 114° 46′; (100) Λ (212) = 121° 49′ (calculated 121° 48′).

X-Ray Diffraction Data

Space Group. $R3$ (C_3^4). The structure of potassium gold dicyanide has been studied by D. T. Cromer, of this laboratory, who will describe it in a publication to appear shortly.

Cell Dimensions. a_0 = 9.74 A.; α = 43.9°; cell volume 403 A.^{3}

Formula Weights per Cell. 3.

Formula Weight. 288.33.

Density. 3.56 grams per cc. (x-ray); 3.55 (pycnometer).

Optical Properties

Uniaxial positive.

Refractive Indices

Wave Length, λ, Microns	n_O	n_E	Birefringence, $n_O - n_E$	$\frac{\lambda}{n_O - n_E}$	Molecular Refraction, Cc.
0.664	1.588	1.689	0.101	6.58	28.4_6
Na 0.5893	1.6005	1.6943	0.0938	6.28	28.8_3
Hg 0.5461	1.6111	1.6996	0.0885	6.17	29.1_6
0.470	1.643	1.713	0.070	6.72	30.0_8
Hg 0.4358	1.6672	1.7219	0.0547	7.97	30.7_6

Colorless.

Dispersion of Birefringence. Potassium gold dicyanide exhibits striking abnormal interference colors. In white light a wedge-shaped fragment of this compound exhibits (in views other than along the optic axis) instead of the usual sequence of interference colors a sequence of purple, green, and white bands. These remain distinct up to the 15th or 20th order. This phenomenon is due to the unusual character of the dispersion of birefringence, which increases rapidly with the wave length, as may be seen from the above table and from the plot on Figure 1. The figures for wave length divided by the birefringence given in the fifth column of the table are proportional to the thickness of the section which gives an interference band of a given order for light of that wave length. This quantity has a minimum in the green at 0.545 micron. For every wave length longer than this minimum (say, in the red) there will correspond a shorter wave length (in the blue), light of these two wave lengths giving an interference band for the same thickness of section. Only green light is transmitted where these two bands coincide. Both red and blue light is transmitted wherever the thickness of the section corresponds to a given multiple of the minimum. This gives rise to a purple band. In the lower orders there is a range of thickness between a given

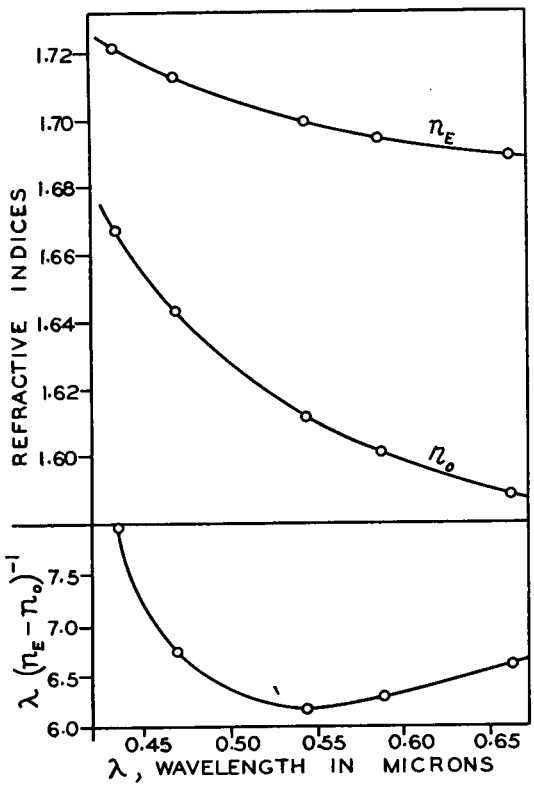

Figure 1. Refringence and birefringence of potassium gold dicyanide as functions of wave length of light

Partial Powder X-Ray Diffraction Pattern of Potassium Gold Cyanide

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
111	8.79	8.72	10
100	6.13	6.10	5
110	5.69	5.67	4
211	4.56	4.53	2
222	4.39	4.38	5
221	4.04	4.03	1
10$\bar{1}$	3.641	3.63	1
210, 120	3.363	3.36	5
322	3.233	3.22	2
11$\bar{1}$	3.131	3.12	8
200	3.066	3.06	10
333	2.929	2.92	2
332	2.920		
220	2.844	2.84	6
321, 312	2.803	...	...
311	2.706	2.70	4
433	2.432	...	...
331	2.417	2.413	6
20$\bar{1}$, 2$\bar{1}$0	2.374	2.372	3
21$\bar{1}$, 2$\bar{1}$1	2.345	2.341	<1
432, 423	2.282	2.275	6
422	2.278		
310, 130	2.241	2.234	3
443	2.240		
444	2.197	2.194	2
320, 230	2.172	2.168	1

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting: λ(Cu$K\alpha$) = 1.5418 A.
[b] Relative peak intensities above background from densitometer measurements.

green band and the purple band of the next-higher order through which all visible light is transmitted.

LITERATURE CITED

(1) Bassett, M., Corbet, A. S., *J. Chem. Soc.* 125, 1660–75 (1924).

WORK done under auspices of Atomic Energy Commission.

108. Potassium Dicopper Tricyanide Monohydrate, $KCu_2(CN)_3 \cdot H_2O$

DONALD I. WALKER[1] and EUGENE STARITZKY,
The University of California, Los Alamos Scientific Laboratory, Los Alamos, N. M.

POTASSIUM dicopper tricyanide monohydrate crystallizes when a saturated aqueous solution of copper cyanide and potassium cyanide is cooled in presence of excess solid copper cyanide. Stability relations of this compound in the system KCN–CuCN–H_2O are given by Bassett and Corbet (*1*).

CRYSTAL MORPHOLOGY

System and Class. Monoclinic, prismatic.

Axial Elements Calculated from Cell Dimensions. $a:b:c$ = 1.484: 1: 0.912; β = 97.5°. Groth (*2*) lists axial elements for this compound which are incompatible with the above set of values but approximate the axial ratios and β determined for potassium

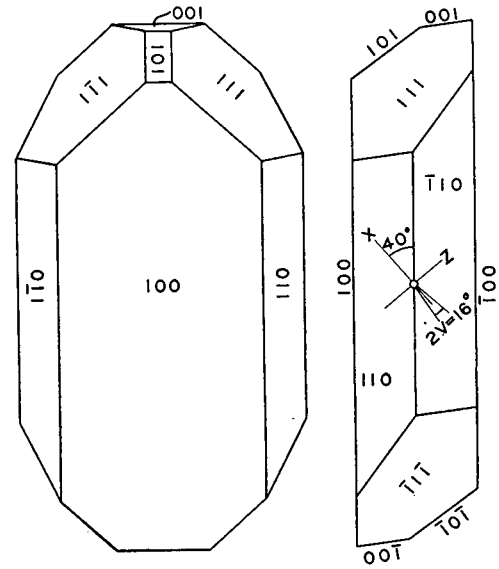

Figure 1. Crystal of potassium dicopper tricyanide monohydrate

Orthographic projections on (100) and parallel to *b*

Partial Powder X-Ray Diffraction Pattern of $KCu_2(CN)_3 \cdot H_2O$

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
100	12.006	11.95	25
110	6.748	6.76	75
200	6.003	6.03	25
011	5.473	5.49	5
$\bar{1}$11	5.172	5.17	15

(*Continued on next page*)

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; λ (Cu$K\alpha$) = 1.5418 A.
[b] Relative peak intensities above background from densitometer measurements.

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Partial Powder X-Ray Diffraction Pattern of $KCu_2(CN)_3 \cdot H_2O$ (*Continued*)

hkl	*d*, A., Calcd.	*d*, A. Obsd.[a]	I/I_1[b]
210	4.835	4.82	30
111	4.806		
$\bar{2}11$	4.254	4.25	15
020	4.080	4.09	20
300	4.002	3.97	5
211	3.863	3.87	35
120	3.862		
002	3.688	3.69	20
$\bar{1}02$	3.662	...	..
310	3.592	3.59	80
021	3.570	...	..
$\bar{1}21$	3.482	3.49	5
102	3.403	...	..
$\bar{3}11$	3.390	...	..
220	3.374	3.37	95
121	3.364	3.35	10
012	3.361		
$\bar{2}02$	3.343		
$\bar{1}12$	3.341		
$\bar{2}21$	3.157	3.17	65
112	3.141	...	..
$\bar{2}12$	3.093	3.09	22
311	3.090		
400	3.002		
221	2.986	2.99	100
202	2.973		
$\bar{3}02$	2.908	...	..
320	2.857	2.858	25
410	2.817	2.814	50

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; λ (Cu$K\alpha$) = 1.5418 A.
[b] Relative peak intensities above background from densitometer measurements.

copper dicyanide. It is probable that Rammelsberg's measurements, from which Groth's figures were derived, were made on crystals of potassium copper dicyanide.

Habit. Blades, flattened {100}, elongated {001}, with {001}, {110}, {101}, {111}.

X-Ray Diffraction Data

Space Group. $P2_1/c$ (C_{2h}^5). D. T. Cromer of this laboratory has determined the structure of this compound. This will be described in a forthcoming publication.

Cell Dimensions. a_0 = 12.11 A.; b_0 = 8.16 A.; c_0 = 7.44 A.; β = 97.5°; cell volume 729 A.^{3}

Formula Weights per Cell. 4.

Formula Weight. 262.25.

Density. 2.39 grams per cc. (x-ray); 2.365 (flotation).

Optical Properties

Refractive Indices (5893 A.). n_X = 1.582 ± 0.02; n_Y = 1.744 ± 0.004; n_Z = 1.748 ± 0.004; geometric mean 1.6895. Molecular refraction 42.4 cc. (based on measured density).

Optic Orientation. $Y = b$; $X\Lambda c$ = 40° with strong inclined dispersion $X_v\Lambda c > X_r\Lambda c$.

Optic Axial Angle (5893 A.). $2V_x$ = 16° with strong dispersion $r > v$.

Colorless.

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(1) Bassett, H., Corbet, A. S., *J. Chem. Soc.* **125**, 1660–75 (1924).
(2) Groth, P., "Chemische Krystallographie," vol. I, pp. 318–19, Engelmann, Leipzig, 1906.

Work done under the auspices of the Atomic Energy Commission.

109. Tripotassium Copper Tetracyanide, $K_3Cu(CN)_4$

EUGENE STARITZKY and FINLEY H. ELLINGER, The University of California, Los Alamos Scientific Laboratory, Los Alamos, N. M.

Tripotassium copper tetracyanide is prepared by evaporating at room temperature an aqueous solution containing potassium cyanide and copper cyanide in the molar ratio 3 to 1. Stability relations of this compound in the system KCN–CuCN–H_2O at 25° C. have been studied by Bassett and Corbet (*1*).

Partial Powder X-Ray Diffraction Pattern of $K_3Cu(CN)_4$

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
110	6.012	6.01	50
$10\bar{1}$	4.832	4.85	15
211	3.839	3.85	65
200	3.766	3.76	100
$20\bar{1}$	3.112	3.10	85
220	3.006	2.991	30
$21\bar{1}$	2.971	2.971	45
222	2.880	2.892	60
$2\bar{1}\bar{1}$	2.790	2.788	95
321	2.474	2.483	70
$20\bar{2}$	2.416	2.414	15
320	2.334	2.340	40
$21\bar{2}$	2.301	2.300	25
$30\bar{1}$	2.242	2.239	15
332	2.092	2.098	20
$32\bar{1}$	2.045	2.046	20
330, 411	2.004	2.005	20
$42\bar{1}$	1.946	1.952	15
410	1.927	1.925	20
400	1.883	1.880	30

[a] Philips 114.6-mm.-diameter powder camera, Straumanis mounting; λ (Cu$K\alpha$) = 1.5418 A.
[b] Relative peak intensities above background from densitometer measurements.

Crystal Morphology

System and Class. Trigonal, trigonal-trapezohedral.

Axial Element. α = 73° 55′.

Habit. Rhombohedrons {110}, occasionally with the base {111}.

Polar Angle. (110) Λ (011) = 77° 7′.

Interzonal Angle. [$1\bar{1}1$] Λ [$\bar{1}\bar{1}1$] = 79° 30′.

X-Ray Diffraction Data

Space Group. $R32$ or $D_3^7(2)$. A strong piezoelectric effect was observed.

Cell Dimensions. a_0 = 8.02 A.; α = 74.1°; cell volume 466 A.^{3} Cox (*2*) reported a_0 = 8.02 A.; α = 77° 32′.

Formula Weights per Cell. 2.

Formula Weight. 284.90.

Density. 2.03 grams per cc. (x-ray); 2.021 (flotation).

Optical Properties

Uniaxial negative.

Refractive Indices (5893 A.). n_O = 1.555; n_E = 1.547; geometric mean 1.5523. Molecular refraction 44.8 cc. (based on x-ray density). Cox and Wardlaw (*2*) give n_O = 1.552 ± 0.003; n_E = 1.544 ± 0.00$_3$.

Colorless.

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(1) Bassett, M., Corbet, A. S., *J. Chem. Soc.* **125**, 1660–75 (1924).
(2) Cox, E. G., Wardlaw, W., Webster. K. C., *Ibid.*, **1936**, 775–81.

Work done under the auspices of the Atomic Energy Commission.

110. Tripotassium Silver Tetracyanide, $K_3Ag(CN)_4$

EUGENE STARITZKY and FINLEY H. ELLINGER,
The University of California, Los Alamos Scientific Laboratory,
Los Alamos, N. M.

TRIPOTASSIUM silver tetracyanide is prepared by evaporating at room temperature an aqueous solution containing potassium cyanide and silver cyanide in the molar ratio 3 to 1. Stability relations of this compound in the system KCN–AgCN–H_2O, at 25° C. have been studied by Bassett and Corbet (*1*), who, however, erroneously assigned to it the formula $K_3Ag(CN)_4 \cdot H_2O$. The silver salt is isomorphous with $K_3Cu(CN)_4$, the composition of which is well established.

Partial Powder X-Ray Diffraction Pattern of $K_3Ag(CN)_4$

hkl	*d*, A., Calcd.	*d*, A., Obsd.[a]	I/I_1[b]
110	6.139	6.14	95
10$\bar{1}$	4.940	4.94	30
211	3.918	3.92	60
200	3.849	3.84	70
20$\bar{1}$	3.181	3.18	35
21$\bar{1}$	3.036	3.04	55
222	2.938	2.947	20
2$\bar{1}$1	2.852	2.853	100
310	2.607	2.598	15
321	2.525	2.529	65
20$\bar{2}$	2.470	2.468	10
320	2.383	2.381	15
21$\bar{2}$	2.352	2.350	10
30$\bar{1}$	2.292	2.295	10
332	2.134	2.140	10
32$\bar{1}$	2.090	2.088	20
411, 330	2.047	2.048	15
400	1.925	1.920	25
420	1.891	1.893	15
3$\bar{1}$2	1.867	1.863	15

[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. Trigonal, trigonal-trapezohedral.
Axial Element. $\alpha = 74° 7'$.
Habit. Rhombohedrons {110}, occasionally with the base {111}.
Polar Angle. (110) ∧ (011) = 76° 55′.
Interzonal Angle. [1$\bar{1}$1] ∧ [$\bar{1}$11] = 79° 22′.

X-RAY DIFFRACTION DATA

Space Group. $R32$ (D_3^7). A strong piezoelectric effect was observed.
Cell Dimensions. $a_0 = 8.19$ A.; $\alpha = 74.2°$; cell volume 497 A.^{3}
Formula Weights per Cell. 2.
Formula Weight. 329.24.
Density. 2.20 grams per cc. (x-ray); 2.18 (flotation)

OPTICAL PROPERTIES

Uniaxial negative.
Refractive Indices (5893 A.). $n_O = 1.521$; $n_E = 1.516$; geometric mean 1.5193. Molecular refraction 45.5 cc. (based on x-ray density).
Colorless.

LITERATURE CITED

(1) Bassett, M., Corbet, A. S., *J. Chem. Soc.* 125, 1660–75 (1924).

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, 3140 South Michigan Ave., Chicago 16, Ill. Work done under the auspices of the Atomic Energy Commission.

CORRESPONDENCE

Analysis of Micron-Sized Particles

Determination of Particle Size

SIR: It has been suggested (*2*) that, in Lodge's Millipore technique for fine particle analysis, it should be possible to derive a relation between the size of the reaction spot, or halo, and the original particle size, as Seely (*7*) and Pidgeon (*6*) had done for the gelatin test for halides. Tufts and Lodge (*8*) have discussed some of the errors inherent in such a relationship. They point out that, if such a calibration is made—for example, for the halide test, using sodium chloride, the error caused by assuming this calibration to hold for all halides of natural atmospheric origin will be less than the other errors of the method. An investigation was made to determine these relationships for several reactions on Millipore.

GENERAL METHOD

The technique used was fundamentally similar to Seely's (*7*). Particles of a known species were collected. The Millipore filter was cut in two; one half was examined directly, and the other half was treated chemically to develop the characteristic reaction spots for the ion under study. Both particles and halos were counted by size classes in identical-sized areas; if the total numbers of halos and particles differed greatly, the sample was rejected, and the experiment was repeated. If this was unsuccessful, study usually revealed that the smallest particles gave no visible reaction spots, and thus the lower limit of identification was established.

The particle and halo counts by size class were then compared. It was assumed that the largest halos were derived from the largest particles, the next largest halos from the next largest particles, etc. Thus, if the tenth largest halo was 50 microns in diameter, the diameter of the tenth largest particle was determined (customarily from smoothed distribution curves).

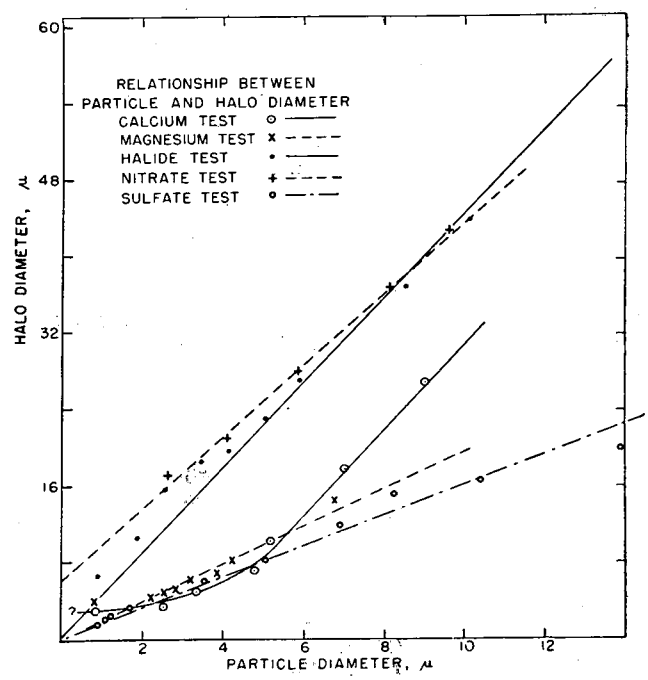

Figure 1. Approximate calibration curves for five reactions on Millipore

Assuming that test substances are representative of their classes

These were said to correspond, and a graph was made of halo *vs.* particle size. If possible, an analytical expression was derived for the relationship. Where this was a straight line through the origin, the slope—i.e., the ratio of halo to particle size—was determined, and was termed the "growth factor." These are shown in Figure 1; details of the determinations are given below.

SPECIFIC TESTS

Halides Other Than Fluoride. The refractive index of sodium chloride, the test material, was found to be so close to that of the filter material that it was difficult to measure the particles accurately. Accordingly, the following method was used, which appears to have general utility in such cases.

A large drop of dry redistilled acetone was placed on a microscope slide, and the half filter bearing the unreacted particles was rapidly placed upon it. When the acetone had evaporated, a clear, cellophanelike membrane remained, with the salt particles embedded in it. This was immersed in distilled water for 0.5 hour, then dried. Thus, air-filled replicas of the particles were obtained which were clearly visible under the microscope.

The mathematical treatment described above yielded the results shown in the figure. The physical evidence indicated that a line through the origin should be expected. This line is shown, and yields a growth factor of 4.73. This value has already been used in several published studies (*3–5*).

Sulfate Ion. Sodium sulfate was used as the test material. Sodium sulfate is weakly anisotropic, and hence the crystals may be distinguished by the use of crossed polaroids. Thus the replica method was not necessary. The best line through the origin yields a growth factor of 1.68.

Nitrate Ion. This was treated in the same manner as the sulfate. Because the reaction spots were radiating clusters of fine needles, frequently unsymmetrical in appearance, the halo diameter used was that of a circle of equal projected area. This is obviously subjective, but after some practice gave reproducible results. No halos were found corresponding to particles smaller than 2.0 microns. This presumably represents the lower limit of identification for this method. The best straight line obviously does not pass through the origin. It has the equation $d_h = 3.73\ d_p + 6.29$, where d_h is halo and d_p is particle diameter.

Magnesium Ion. It was necessary to use dry, powdered magnesium sulfate in order to obtain dry crystals for examination. Fading of the halos was retarded by not washing the filter after chemical treatment. The growth factor was found to be 1.88.

Calcium Ion. Here again it was necessary to use a dry powder spray. The test substance was calcium acetate. The halo-particle relationship does not appear to be a simple one.

CONCLUSIONS

It is possible to determine particle size spectra for a number of chemical species. It seems possible that this technique could be extended further to other ions. Field tests have shown that the halide technique yields highly satisfactory results, giving the expected logarithmico-normal distribution, which also obeys Junge's (*1*) "*r*-cube" law.

ACKNOWLEDGMENT

The authors wish to thank Horace R. Byers and Roscoe R. Braham, Jr., of this laboratory for many helpful discussions.

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(2) Lodge, J. P., ANAL. CHEM. **26**, 1829 (1954).
(3) Lodge, J. P., *J. Meteorol.* **12**, 493 (1955).
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(5) Lodge, J. P., McDonald, J. E., Baer, F., *Ibid.*, **11**, 318 (1954).
(6) Pidgeon, F. D., ANAL. CHEM. **26**, 1832 (1954).
(7) Seely, B. K., *Ibid.*, **24**, 576 (1952).
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MEETING REPORT

Society for Analytical Chemistry

A JOINT meeting of the Microchemistry Group of the society with the Mid-Southern Counties Section of the Royal Institute of Chemistry was held in Southampton Oct. 7, at which the following papers were presented and discussed.

Trace Elements in Archeology. C. F. M. FRYD, Department of Government Chemist, Government Laboratory, Clements Inn Passage, Strand, London, W. C. 2.

Recent extensions of public interest in archeology have been accompanied by a widening of the scope of the analyst in the examination of archeological specimens. The archeologist today relies on the chemist not only to help him in the exposure of occasional fraud, but much more frequently to assist in the dating of a specimen, either directly when a time scale can be established for the accretion or removal of an element, or comparatively when one specimen can be presumed by reason of intermediate chemical characteristics to fall chronologically between two known specimens. The trace elements are important in this respect and their examination is not without interest.

Methods for Determining the Trace-Element Status of Plants. E. J. HEWITT, University of Bristol, Agricultural and Horticultural Research Station, Long Ashton, Nr. Bristol.

It is possible to recognize changes from the optimum states of trace-element availability for plants in three ways—by soil analysis, by plant analysis, and by physiological studies. The first was not considered further. Physiological studies may be partly visual, but they require detailed knowledge of plants; the symptoms of deficiency may be confused by other disorders and be already serious by the time they could be seen. Alternatives were fertilizer trials, pot cultures, or spraying or injecting trace elements.

Plant analysis is usually made on physiologically active regions of the specimen. If stringent precautions are taken to avoid contamination, especially from soil, reasonably consistent results are obtained by different analysts, except for boron and iron. One difficulty is that the metal content may vary considerably with the portion of the plant taken; another is that plants could show deficiency symptoms for a particular metal but might contain not very much less than a healthy plant—the diagnostic value of chemical analysis is then not great.

Work in his own department had indicated particular difficulties that might arise with iron.

Estimation of Trace Elements in Plant Material and Soils by Means of *Aspergillus niger*. D. J. D. NICHOLAS, University of Bristol, Agricultural and Horticultural Research Station, Long Ashton, Nr. Bristol.

The mold *Aspergillus niger* has been used as a test organism to determine copper, zinc, manganese, and molybdenum in soils, plant and animal tissues, and in enzymes. Experimental methods have been developed for the preparation of standard growth series for iron, zinc, copper, manganese, and molybdenum. The pure culture methods include purification of culture solutions by chemical or ion exchange procedures and the preparation of glassware and inocula free from trace metals. The bioassay method is preferred to chemical procedures because of its higher accuracy and specificity at low concentrations of the metal.

At a meeting of the Physical Methods Group on Nov. 30 the following officers were elected: chairman, J. E. Page; vice chairman, R. A. C. Isbell; honorary secretary and treasurer, L. Brealey, 417 High Road, Chilwell, Notts.

At a meeting of the Midlands Section on Dec. 7 in Birmingham, the following paper was presented.

Some Physical Methods for Analysis of Phosphorus Compounds. D. E. C. Corbridge, Research Department, Albright & Wilson, Ltd., Oldbury, Birmingham.

Methods were presented for identification and analysis of phosphorus compounds using x-ray diffraction and infrared techniques. The correlation of infrared absorption with molecular structure in the use of such data for analytical purposes was discussed. The potassium bromide disk technique and its application to the quantitative analysis of condensed phosphates were described. X-ray powder diffraction data for a number of phosphorus compounds were also presented.

A joint meeting of the Association of Public Analysts and the Society for Analytical Chemistry, organized by the Western Section, was held Dec. 10 at Cardiff. A paper on "Sucrose Loss from Ice Cream on Storage," by H. J. Evans, W. Kwantes, D. C. Jenkins, and J. I. Phillips, Public Analyst's Office, Carmarthen and Area Pathological Laboratory, Carmarthen, was presented.

From the results of experimental work it was concluded that the diminution in content of sugar in certain samples of ice cream during storage is attributable to the presence of the diplococcus *Leuconostoc mesenteroides*. This organism is well-known for its ability to convert sucrose into dextran. The extent to which this change occurs would obviously depend upon many factors, the main ones being the time and temperature of storage and the number of organisms initially present.

Experiments had shown that a considerable change in the concentration of sucrose originally present in the ice cream might occur during a few days' storage at 70° F., and even the temperature of a normal domestic refrigerator was not sufficiently low to prevent slow diminution in the sugar content.

When ice cream was stored below 28° F., there was no evidence that changes in the concentration of sucrose occurred, but at this temperature only a very few trials had been made. It was known that manufacturers of ice cream stored their products for many months in deep-freeze refrigerators without any apparent change in composition.

It was recommended that samples of ice cream intended for determination of their sucrose content should preferably be analyzed while still frozen solid and certainly should not have been kept more than a few hours at room temperature before the determination of sugar was begun.

At a meeting of the Scottish Section held Dec. 14 in Edinburgh a paper on "Statistics for Chemists, Statistical Control in Chemical Analysis," was presented by B. Woolfe, Institute of Animal Genetics, University of Edinburgh.

For the chemical analyst, statistical control is primarily a labor-saving device, telling him when and to what extent replicate analyses are necessary, guarding him against wasting time in pursuit of greater accuracy than is needed for the purpose in hand, and preventing him from harboring optimistic illusions about the reliability of his results. When routine analyses are conducted for process control and the like, statistical control will give early warning of any impending trouble.

The basic statistical theory involved is simple. A typical analysis starts with the quantitative operation of weighing out or measuring the sample, followed by a series of manipulations, and ends with a chemical or physical measurement. Each step has its characteristic degree of nonreproducibility or error, and the dubiety of the final result will be the resultant of all these errors acting jointly. Almost all the problems arising in chemical analysis can be solved from the principle of the additive nature of variance. This principle was demonstrated, and examples were given of its use.

For many practical purposes, a statement of the average composition of the material analyzed is not enough. Information is also needed about the variability of heterogeneity of the material. This information rarely appears in the analyst's report, though his notebook often contains the requisite data. The problem of sampling variable material was discussed, and the importance was emphasized of having due regard to the purpose the analysis is designed to serve.

The 11th annual general meeting of the Biological Methods Group was held Dec. 9 in London, at which the following officers were elected: chairman, K. L. Smith; vice chairman, S. A. Price; honorary secretary and treasurer, K. A. Lees, Glaxo Laboratories, Sefton Park, Stoke Poges, Bucks. The following papers were presented.

Microbiological Plate Assay of Penicillin in Compound Feeding Stuffs. J. S. Simpson and K. A. Lees, Glaxo Laboratories, Ltd., Stoke Poges, Bucks.

A microbiological plate method was described for the assay of penicillin in feeding stuffs with *Sarcina lutea* as test organism. The method is capable of detecting small quantities of penicillin and can be used for the assay of samples containing less than 5 units per gram of penicillin; at this level, plate assay methods using *Bacillus subtilis* are insensitive.

Samples are extracted with 25% v./v. acetone buffer solution and are diluted to levels of 0.1 and 0.05 unit per ml. The solutions are applied to the usual cups in a randomized manner using the 8 × 8 quasi-Latin square design. For purposes of strict comparison the reference standard solutions of penicillin are prepared by dilution in the sample solvent solution.

The limits of error ($P = 0.95$) of the method are of the order of 90 to 112% when six samples and two standards at two levels each are employed on the 8 × 8 quasi-Latin square plate.

Simple Method for Determination of pA_2 at 2 Minutes. Mary F. Lockett, Department of Physiology, Chelsea Polytechnic.

H. O. Schild [*Brit. J. Pharmacol.* **2**, 189 (1947)] introduced pA_x as a measure of the intensity with which an inhibitor drug competes with a naturally occurring key drug for the occupation of specific cell-surface locks or receptors.

A dose of key drug, k, which produces a response about 50% of maximum, is allowed to act every 3 minutes, for 30 seconds, on a strip of guinea pig ileum suspended in a known volume of Tyrode's fluid at 32° C. When the responses to k are constant, a dose of inhibitor is added exactly 2 minutes before the next dose of key drug is due. At the end of the 2 minutes, double the usual dose, $2k$, of key drug is added; 30 seconds later, both drugs are washed out together. Return is then made to dose k. When the effects of the inhibitor have worn off and constant responses are again recorded to k, another trial may be made with inhibitor.

Three weights of inhibitor are chosen, A, B, and C; these increase as simple powers of two or three. In each case, the difference between the response to $2k$ inhibited, and the mean of three preceding control responses to k uninhibited, is expressed as a plus or minus percentage of that mean. Average percentages, y, plotted against the log-dose of inhibitor, x, fall on a straight line. This line is calculated by the method of least squares: $b(\text{slope}) = S^2/n(\bar{x} - x)^2$, and the standard error of the mean $= \sqrt{S^2/N}$. The standard deviation (S_y) of any value of y will be given by the equation:

$$S_y = S\sqrt{\frac{1}{N} + \frac{(\bar{x} - \bar{x})^2}{\Sigma n(x - x)^2}}$$

The value S_y, and the fiducial limits of y at the value of x corresponding to pA_2—i.e., x when $y = 0$—are determined.

Values of x, when $y = 0$, and for these limiting values of y, are expressed as molar concentrations resulting from their addition to bath volume. The negative logarithms to the base 10 of these molar concentrations afford pA_2 values.

Estimates of pA_2 so determined are in close agreement with those recorded by Schild, and the fiducial limits ($P = 0.05$), obtained by this method, have not exceeded ±3%.

Automatic Apparatus for Isolated Preparations, Suitable for Assay of Oxytocin and Similar Assays. J. A. Lock, Research and Biological Department, Crookes Laboratories, Ltd., Park Royal, N. W. 10.

An apparatus was demonstrated which is basically similar to those previously described, but has the practical advantage that doses of drug are added directly to the isolated organ bath by adjustable relay-controlled syringes.

This arrangement overcomes the difficulty which frequently arises when the drug is incorporated in reservoirs of bathing fluid: Trial doses added directly to the bath, when extrapolated to the necessary dilutions in the bathing fluid, are not always found effective, and the reservoir may have to be emptied and refilled several times to achieve a satisfactory result. Further, the preparation may vary in sensitivity during the course of an assay; if this occurs, the assay may have to be abandoned. In the arrangement demonstrated, adjustments of doses may be carried out rapidly over a relatively wide range with no disturbance to the rest of the apparatus.

At a meeting of the Midlands Section on Dec. 15, R. Belcher, Department of Chemistry, The University, Edgbaston, Birmingham, discussed the advances in quantitative inorganic analysis during the past 2 years under the headings of primary standards, indicators, titrants, reagents, and established methods.

Evaluation of Paper Chromatograms by Direct Polarographic Scanning

Alois Langer, Westinghouse Research Laboratories, East Pittsburgh, Pa.

DURING a polarographic study of solid microelectrodes of the gold, platinum, or gold-mercury amalgam type (*2*), useful curves were obtained by diminishing the amount of supporting electrolyte and having the solid electrode just touch a filter paper moistened with the electrolyte being investigated. Relatively slow scanning was used, so that the resulting current-potential curves resembled the shape of a normal polarographic wave (*4*). Thus it was found that by placing the filter paper on a porous porcelain block partly immersed in the saturated potassium chloride solution of the calomel electrode, and using an amalgamated gold bead as the cathode, polarographic spot test evaluations could be made. The encouraging results of these tests led to the construction of a simple apparatus for the continuous evaluation of paper chromatograms with inorganic or organic spots (Figure 1).

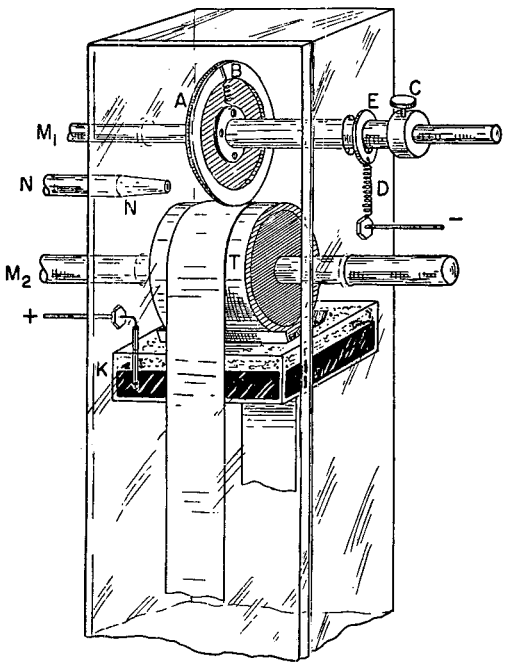

Figure 1. Polarographic scanner for paper chromatograms

The polarizable electrode is a mercury amalgamated 40-mil gold wire, *A*, wound tightly in a shallow groove of a plastic wheel about 3 inches in diameter and held tightly by the tension of a small spring, *B*. The annealed gold wire was amalgamated by immersing it in a mercury pool and brushing slightly. The wheel can be rotated slowly by a 1-revolution-per-hour motor, M_1, attached through a flexible coupling. It can be detached from the motor drive by loosening screw *C*, thus allowing it to rotate freely on its shaft, driven only by contact with the filter paper on roll *T*. Sidewise adjustments of the wheel can be made by sliding it along the shaft. Vertical adjustment was allowed for by the use of slotted bearings. This allowed the wire to be pressed against the filter paper by adjusting the tension of spring *D* attached to wiper *E*, which also served as the electrical connection for the wire electrode.

For the nonpolarizable electrode, a saturated calomel half cell is used. The container, *K*, is made of plastic. Glass wool is immersed in the potassium chloride solution and exposed through a slot in the cover to a porous porcelain tube, *T*, about 1.5 inches in diameter and several inches wide. This tube is supported on its shaft by rubber stoppers so that it can be rotated at low speed (about $^1/_5$ r.p.m.) by motor M_2. The filter paper is supported by the drum and passed slowly under the amalgamated gold wire.

The whole unit is enclosed in a tight, transparent box with a removable front to facilitate the placing of the filter paper. Air in the box can be replaced by purified nitrogen through nozzle *N* to eliminate the interference of dissolved oxygen with the polarographic process.

The nitrogen is first moistened by bubbling through bottles containing water; some wet glass wool is also placed at the bottom of the box to keep the atmosphere saturated with water vapor. In this way, drying out of the filter paper and evaporation of the potassium chloride solution are minimized.

EXPERIMENTAL

Many experiments were made under varying conditions, mainly to establish the feasibility of the procedure rather than to find the optimum conditions for a given problem. First, individual droplets of solution ranging in cation concentration from 0.1*M* to 0.01*M* were placed on the filter paper strip, which had been previously moistened with the supporting electrolyte and blotted to remove the excess. Potassium chloride, potassium nitrate, ammonium nitrate, and others in 0.1*M* or 0.01*M* concentrations were used as supporting solutions. In a limited number of actual chromatograms, the buffer solution used as a migration medium served also as the supporting electrolyte.

To determine the presence of a reducible substance on the moistened paper, the polarizing potential of the wire electrode was made as negative as the amalgamated electrode would allow, without having too high a background current. Depending on the electrolyte, this was about −1.6 to −1.8 volts. In all cases, the useful range of potentials for the amalgamated electrode was lower than for the mercury dropping electrode with the same electrolyte. When the paper was scanned by rolling it between the wire electrode and the porcelain tube, a current was recorded as soon as the wire made contact with a spot containing a reducible substance. The Sargent Model XXI recording polarograph was used for recording. Because the recorder paper moved continuously with time while a constant polarizing potential was maintained, individual spots were recorded as peaks. After a more or less sudden rise, the tops of the peaks corresponded to the size of the spot; they were usually not flat but showed some fluctuations as indicated in Figure 2,*A*. The fluctuations may be due to unevenness of the filter paper (therefore, a variable contact area) more than to concentration variations across the drop, because they can be minimized by the adjustment of the tension of spring *D*. For quantitative estimation of the ion concentration, it was found that the average current is roughly proportional to the concentration over a wide range. This was shown when spots of a confined area were made with different concentrations of the solutions and care was taken to prevent an evaporation of the supporting solution. However, only a rough estimate of the amount could be made from average peak height and peak top length, because the width of the spot area was not considered.

When the movement of the filter paper was stopped at these peaks and a current-voltage curve taken with a stationary electrode or by rotating the wire with motor M_1 to expose new surface of the wire to the paper, current-potential curves were obtained. These waves closely resembled the ones obtained with the mercury dropping electrode but did not have the characteristic oscillations. Although this is a procedure to which objections can be raised (*1*), some useful information can be obtained. Here,

too, the half-wave potentials were a qualitative indication of the reducible ion present in the spot. Shifts in the half-wave potentials of the order of 0.2 volt or more were observed, but these may be partly due to the varying IR drop depending on the moisture content of the filter paper. No detailed experimental evaluation of these phenomena was made. Normal waves were usually obtained but sometimes maxima were indicated (Figure 2,B). These maxima usually were not so pronounced as those with solid microelectrodes in a liquid medium, but showed some of their peculiar behavior, such as occasionally not maturing under otherwise identical conditions.

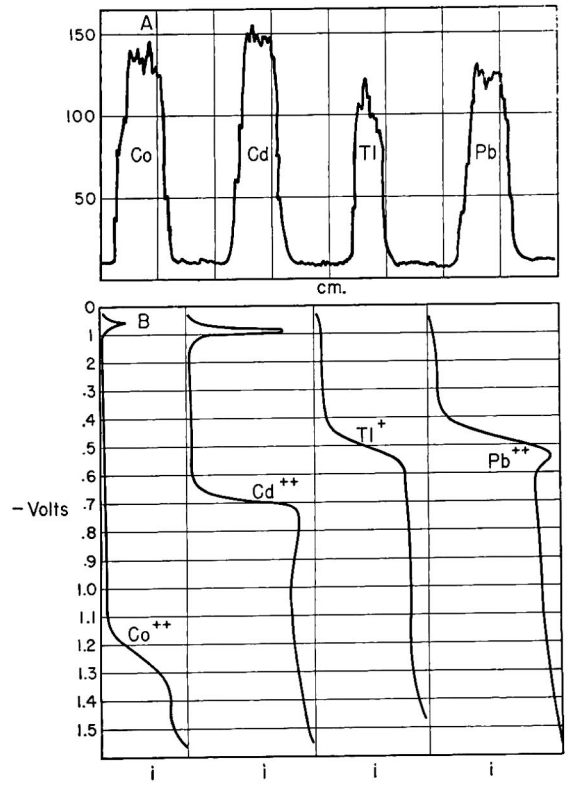

Figure 2. Polarographic curves

A. Current peaks obtained by scanning at −1.5 volts. Whatman filter paper impregnated with 0.1M KNO_3 and with drops of 0.1M nitrate solutions of the indicated cations.
B. Current-voltage curves at these peaks with a stationary electrode

Attempts to evaluate half-wave potential by derivative polarography were tried by winding two wire electrodes side by side on drum A, but separately connected to polarizing potentials. Usually, the derivatives curves were much too distorted to be of any use.

The procedure described was applicable not only to inorganic ions but to the many organic compounds which can be evaluated polarographically. Runs were made successfully with nitrobenzene, nitrotoluene, and other nitro compounds in alcohol-water solutions. The method described thus resembles other scanning procedures for evaluation of paper chromatographs by use of photometry, radioactivity, fluorescence, and others (*3*).

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(3) Lederer, E., Lederer, M., "Chromatography," Elsevier, New York, 1953.
(4) Rodgers, L. B., Miller, H. H., Goodrich, R. B., Stehney, A. F., ANAL. CHEM. 21, 777 (1949).

Modified Thermocouple for Peak Exotherm Measurement

John F. Palmer, Jr., and Robert S. Barnes, Monsanto Chemical Co., St. Louis, Mo.

A MINIATURE-SIZE thermocouple has been successfully adapted to the measurement of the peak exotherm temperature exhibited by unsaturated polyester resins undergoing catalytic cure. The need for a miniature thermocouple centers around the necessity of avoiding underestimation of the peak exotherm temperature in the preparation of castings of polyester resins. For example, in the preparation of a 100-pound casting containing 25% glass and having a specific heat of 0.32 (Sonneborn, R. H., "Fiberglas Reinforced Plastics," 1st ed., p. 116, Reinhold, New York, 1954) an error of 40° F. underestimation of peak exotherm temperature would result in 1280 B.t.u. of unexpected heat being transmitted to the mold. This error might have occured in the small sample determination because of the larger heat capacity and slow response of the temperature sensing element. This could have caused a very serious situation to exist if proper means had not been previously provided for the removal of this extra heat load.

Work with the gel time and peak exotherm procedure, as outlined in Military Specification **MIL-R-7575A,** April 27, 1953 [Smith, A. L., Proceedings of Sixth Annual Technical Session, Reinforced Plastics Division, Society of Plastics Industry, Inc., Sec. 1, p. 3 (1951)] led to an investigation of thermocouples permitted by the specification. Most satisfactory results have been obtained with a miniature iron-constantan thermocouple fabricated into a No. 18, 2-inch-long, stainless steel hypodermic needle. The couples themselves were prepared from 30-gage enameled and cotton-covered iron and constantan thermocouple wire with the junction silver soldered into the tip of the hypodermic needle. The ferrule of the hypodermic needle was, in turn, soldered to the end of a piece of $^3/_8 \times 4$ inch brass tubing which served as a handle.

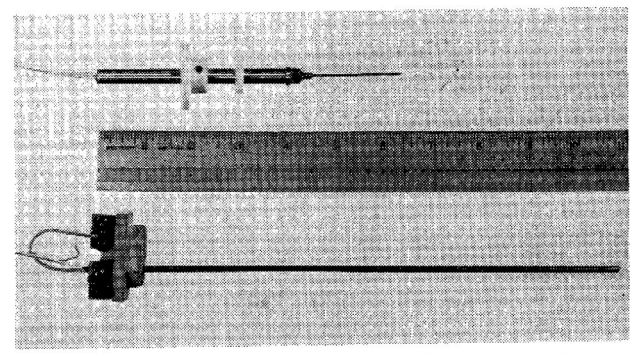

Figure 1. Comparison of hypodermic needle thermocouple and rodlike commercial couple

To facilitate uniform placing of the thermocouple in the test tube, a centering ring and plug cap were fabricated from Teflon and attached to the upper shank of the thermocouple (Figure 1). Temperatures were recorded by a single-pen Leeds & Northrup Speedomax Type G strip recorder modified to have a linear chart speed of 1 inch per minute.

These small thermocouples, as a result of the radically reduced mass of metal in contact with the test sample, conduct less heat away than conventional couples and thus indicate a higher temperature, this being closer to the true peak exotherm temperature that would be reached in a mold. The lower mass of the thermocouple also results in more rapid response to temperature changes, thus more accurately indicating gel and cure time.

In Table I values for gel and cure time and peak exotherm temperature are given for two commercial polyester resins, each catalyzed, respectively, with 0.5, 1.0, and 2.0% by weight of benzoyl peroxide. The catalyst was added as a 50% paste of benzoyl peroxide in tricresyl phosphate, Luperco ATC. Five samples of each resin at all three catalyst concentrations were tested with both the miniature hypodermic needle thermocouple and a 1/8-inch commercial iron-constantan thermocouple. The thermocouples were alternated on each successive individual test to minimize any possible development of a pattern. The two thermocouples were compared at various temperatures and were found to agree exactly when measuring the temperature of a large mass.

Table I. Comparative Exotherm Data

Catalyst Concn., Added Benzoyl Peroxide, %	Hypodermic Needle Thermocouple			Commercial Thermocouple		
	Time, Min.		Peak exotherm, ° F.	Time, Min.		Peak exotherm, ° F.
	Gel	Cure		Gel	Cure	
Resin A						
0.5	3.8	6.5	419	4.2	7.0	376
	3.8	6.5	419	4.3	7.0	379
	3.8	6.5	421	4.2	7.0	376
	3.7	6.3	419	4.2	6.8	381
	3.8	6.5	419	4.2	6.8	383
Av.	3.8	6.5	419	4.2	6.9	379
1.0[a]	2.7	4.3	439	2.7	4.3	401
2.0[a]	1.8	3.1	453	1.6	2.8	414
Resin B						
0.5[a]	8.3	11.2	408	10.1	12.3	378
1.0[a]	4.9	7.0	421	5.5	7.0	394
2.0[a]	3.0	4.6	435	2.7	4.0	401

[a] Arithmetic average of 5 consecutive tests.

In Table I, the data show that the hypodermic needle thermocouple consistently gave peak exotherm temperatures 30° to 40° F. higher than a commercial 1/8-inch couple.

Individuals having occasion to use any system employing fairly large masses of polyesters, and desiring more accurate measurement of the peak exotherm temperature and resin characteristics, may find good use for this thermocouple of improved design, reproducibility, and accuracy.

Preservation of Ninhydrin-Amino Acid Chromatograms

Gunter Zweig, Charles F. Kettering Foundation, Yellow Springs, Ohio

In order to preserve ninhydrin-treated paper chromatograms, Kawerau [Kawerau, E., and Wieland, T., *Nature* **168**, 77–78 (1951)] recommended dipping the papers first into a copper nitrate solution and then into a chloroform solution of methyl methacrylate.

The following modification of this technique was found to be successful.

The ninhydrin-treated chromatogram is dipped into dilute copper nitrate (1 ml. of saturated aqueous copper nitrate and 0.2 ml. of 10% volume by volume nitric acid, diluted to 100 ml. with ethyl alcohol). The papers are then quickly neutralized in vapors of ammonia and are air dried. The papers are then sprayed with Krylon permanent crystal clear acrylic spray, available as an aerosol bomb (Krylon, Inc., Philadelphia 46, Pa.). The chromatograms, thus treated, are stable without any further precautions for months.

Aerosol Chromatographic Spray

Gunter Zweig, Charles F. Kettering Foundation, Yellow Springs, Ohio

For the visualization of amino acid chromatograms a ninhydrin spraying or dipping solution has been commonly employed (Block, R. J., Durrum, E. L., and Zweig, G., "Paper Chromatography and Paper Electrophoresis," Academic Press, New York, 1955). For small sheets (8 square inches) the dipping technique has been found satisfactory. Large sheets ($18\frac{1}{4} \times 22\frac{1}{2}$ inches), however, can be sprayed more easily.

Several models of sprayers for chromatography are commercially available or can be made at home (Block, R. J., Durrum, E. L., and Zweig, G., "Paper Chromatography and Paper Electrophoresis," Academic Press, New York, 1955). In order to obtain a uniform spray, a source of compressed air should be available. Furthermore, if working with a number of different spraying reagents simultaneously, it would be cumbersome to empty, clean, and refill the sprayer each time when changing solutions. Therefore, a cheap, disposable and portable spray gun is to be desirable. An aerosol bomb seemed to satisfy these conditions.

Aerosol-ninhydrin bombs for spraying large amino acid chromatograms have been used by this foundation. Excellent results were obtained with ninhydrin dissolved in acetone or *n*-butyl alcohol. The bombs were prepared by Aeropak, Inc., Chicago 37, Ill., and may be obtained from Scharr & Co., Chicago, Ill.

The foundation is planning to design other aerosol chromatographic sprays to be used for chromatograms of sugars, organic acids, keto acids, steroids, and other compounds.

Simplified Isoteniscope

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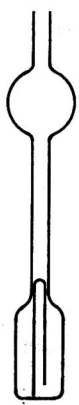
A simplified apparatus equivalent to the Smith-Menzies isoteniscope [Smith, A., and Menzies, A. W. C., *J. Am. Chem. Soc.* **32**, 1412 (1910)] was developed to obviate the difficulty of filling the classical isoteniscope with viscous organic liquids. It can also be used satisfactorily for any system in which the substance under investigation is the confining liquid.



The liquid is pipetted into the lower reservoir (open leg), then the sealed leg (a piece of glass tubing 7 mm. in diameter and 50 mm. long) is dropped in. The apparatus is connected to a standard manometric system and thermostated. Air, trapped in the sealed leg which rests against the bottom of the reservoir, forces the liquid level in the sealed leg below that in the larger open leg. As the apparatus is brought to temperature and moderately evacuated, the level in the sealed leg will fall; a stream of bubbles will rise from the open end of the sealed leg and pass through the open leg. Trapped air is thus removed and the liquid boiled out. Liquid levels in both legs are brought to the same height and the pressure noted. The boiling out procedure is repeated until agreement between readings is obtained.

Values of vapor pressures obtained with the modified and classical isoteniscope agree within experimental error. A further advantage of the modification is that gentle boiling stirs the liquid and ensures rapid temperature equilibration between bath and liquid.

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