ANALYTICAL CHEMISTRY

WALTER J. MURPHY, Editor

Bigger and Better

TEN half-day sessions featuring 183 technical papers are on the schedule of the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy. The meeting will be held at Pittsburgh, February 28 through March 4, and represents the sixth joint conference of the Pittsburgh Section of the AMERICAN CHEMICAL SOCIETY and the Spectroscopy Society of Pittsburgh.

In addition to the large number of technical sessions, the conference will also feature meetings of several ASTM committees concerning absorption and emission spectroscopy, a meeting of the National Research Council–National Bureau of Standards Committee on Ultraviolet and Visible Spectral Data, exhibits by 37 equipment manufacturers, an employment bureau, and a technical and scientific book center. Also on the schedule is the informal discussion period which has proved popular in the past. The subject this year will be x-ray fluorescence spectroscopy.

The conference dinner will feature an address by Alvin M. Weinberg, director of the Oak Ridge National Laboratory, who will speak on the subject of "Future Goals for Large Scale Research."

The scope of the subjects covered in the technical sessions is large, as indicated by the abstracts (pages 308 to 329). They include organic, metal and alloy analyses, polarography, radiochemical methods, x-ray, infrared, and ultraviolet spectroscopy, mass spectrometry, and flame photometry.

In looking over this program and recalling earlier conferences, we are amazed at its rapid growth. The first such meeting, held in 1950, lasted 3 days, featured 56 papers, and had what was considered to be a surprisingly high registration of 767. Attendance now is on the order of 2000. In 1950, there were 14 exhibiting companies. In 1955, 37 will participate.

The Pittsburgh Conference, the summer symposium, and other special meetings, plus the Analytical Division sessions at the ACS national meetings, indicate an active and growing interest which augurs well for the future of analytical chemistry.

In Unity There Is Strength

THE old adage that in unity there is strength was well illustrated in a recent tax case involving the professional status of an analytical chemist.

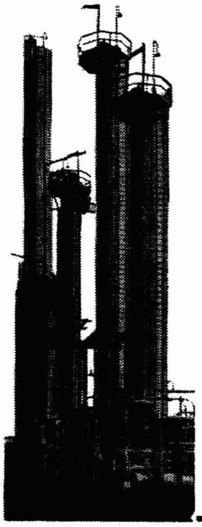
To chemists and chemical engineers it is axiomatic that chemistry is a profession. In the case at hand, however, it took nine years for a chemist, aided by counsel and expert witnesses secured by the AMERICAN CHEMICAL SOCIETY, to establish his professional status.

The story, as outlined elsewhere in this issue (page 28 A), centers about Abraham Mirkin, analytical chemist and ACS member, who owns and operates the Mirkin Analytical and Pathological Laboratory in New York.

In 1946 he sought the same tax exemption on an unincorporated business which is allowed other professional men engaged in the practice of their professions. New York tax authorities rejected his claim. This led to a long legal battle, which finally ended with a decision supporting Mirkin's position.

Without the support of the ACS with its more than 70,000 members, chemists and chemical engineers, individually or in small groups, would often find it difficult if not impossible to fight effectively for professional recognition. This is particularly true where long-drawn-out and expensive litigation is involved. This has happened in the past and can happen again.

The AMERICAN CHEMICAL SOCIETY participated in this case as it has in other legal cases in which professional recognition of chemists and chemical engineers was an issue. We are happy to report that, in all these instances, professional status was recognized. These constitute a very practical reason why *every* chemist should be a member of the ACS.



Advances in

Separation of Hydrocarbons and Related Compounds

Four papers from the Symposium on Advances in Separation of Hydrocarbons and Related Compounds, presented before the Division of Petroleum Chemistry at the 126th meeting of the AMERICAN CHEMICAL SOCIETY, New York, N. Y. Other papers in this symposium are published in the February issue of *Industrial and Engineering Chemistry.*

Separation and Analysis of Gases and Volatile Liquids by Gas Chromatography

H. W. PATTON, J. S. LEWIS, and W. I. KAYE

Research Laboratories, Tennessee Eastman Co., Division of Eastman Kodak Co., Kingsport, Tenn.

Gas chromatographic techniques were used to study the potentialities of selective adsorption and partition methods for the separation and analysis of gases and volatile liquids. Methods involving elution by hydrogen, nitrogen, or carbon dioxide from columns containing charcoal, silica gel, or alumina provided excellent separation of hydrocarbons and other nonpolar materials. Gas chromatographic elution is simple, accurate, and very effective for the identification and determination of the components of many gaseous and liquid mixtures, particularly of hydrocarbons.

IN RECENT years the separation of materials by selective sorption, partition, and ion exchange processes has been developed into a very powerful and versatile tool for analysis and purification. Although many variations on the fundamental technique have been made and the scope of the method has been tremendously broadened, most of the work reported has involved samples of relatively nonvolatile materials in volatile liquid solvents. Some methods involving a gaseous phase have been used during the last decade, but this aspect of chromatography is still relatively new.

The techniques and principles of gas chromatography are similar in many respects to those of the more common column procedures, except that the mobile phase is gaseous rather than liquid. In brief, the usual procedure is as follows: The material to be separated is adsorbed in a narrow band on one end of a column of adsorbent such as activated charcoal. An adsorbent or inert support coated with a high-boiling liquid is advantageous in some cases (gas-liquid partition). The sample is caused to move through the column by application of heat, passage of a carrier gas through the column (elution), passage of a carrier gas containing a strongly adsorbed vapor through the column (displacement), or some combination of these methods. When it is possible to arrange conditions that produce sufficiently different rates of migration, the components are separated and can be detected or collected as they emerge from the column.

The separation of gases and vapors by chromatographic methods has been reported by a number of workers. Hesse and Tschachotin (*10*), Cremer and coworkers (*4–6*), and Janak (*13*) described techniques in which the sample materials are eluted from an adsorbent by a carrier gas. Instead of an adsorbent, Martin and James (*11, 14*), Phillips and coworkers (*9, 12, 15*), Cropper and Heywood (*7*), and Ray (*16*) used columns packed with granules of a support covered with a high-boiling liquid. Development by displacement has been described by Claesson (*3*) and Phillips (*9, 12*). Turner (*18*) described an apparatus in which hydrocarbon gases were separated on a charcoal column as they were moved through it by a heater. A later modification employing displacement by a vapor and other changes is available commercially (*2*). Glueckauf and coworkers (*8*) obtained some isotopic enrichment when neon was passed through activated charcoal at low temperatures. Wirth (*19*), Turkel'taub (*17*), and Barrer and Robins (*1*) have published the results of relevant investigations.

Exploratory investigations of adsorption methods (elution and displacement from adsorbents) and the gas-liquid partition method (elution from liquid-covered granules) were made in this laboratory. Because of the early discovery that the method involving elution from adsorbents was readily adaptable to the accurate determination of light hydrocarbons and some of the gases associated with them, the work was mainly concentrated on this technique. This paper is largely concerned with the separation and analysis of these gases and volatile liquids by elution from columns of charcoal, silica gel, and alumina.

EXPERIMENTAL

Procedure. The general arrangement of the apparatus is shown by the block diagram in Figure 1.

A stream of carrier gas passes through the system continuously during a run. The flow rate is indicated by a flowmeter and controlled by a needle valve. Constant pressure is maintained at the input to the flowmeter by allowing excess carrier gas to escape through a constant head of water. When the detector is a thermal conductivity cell, the carrier gas passes through its reference channel before entering the column. Gas samples are handled in a glass vessel provided with a bypass through which the carrier gas passes while the system is being prepared for a run. Liquid samples are injected onto the end of the column through a rubber serum cap with a hypodermic syringe. The carrier gas causes each sample component to move through the column at a rate that depends on how strongly it is held by the adsorbent. In favorable cases, the mixture is separated and each component passes from the column into the sample channel of the detector at a different time. When it is desirable, the separated fractions are collected for investigation by infrared absorption or other means.

Carrier Gases. The choice of carrier gas depends to a large extent on the materials to be analyzed. With a thermal conductivity detector, the sensitivity to a given substance can be changed tremendously by using different carrier gases. Of course, there is no response from that portion of a sample that is the same as the carrier. Nitrogen is a satisfactory carrier for many separations, but the response of carbon monoxide and the C_3 and C_4 hydrocarbons in nitrogen is rather poor. A system using hydrogen as carrier is much more sensitive to hydrocarbons heavier than methane. The explosion hazard and the possible hydrogenation of unsaturated materials are obvious disadvantages. Carbon dioxide has been used with some success for the determination of oxygen, nitrogen, hydrogen, and the hydrocarbons through C_2. The stability of the thermal conductivity cells is excellent with hydrogen, good with nitrogen, and poor with carbon dioxide.

The flowmeter is of a conventional type that measures the pressure differential developed across a capillary. The efficiency of separation is not critically affected by moderate changes in the flow rate. Increasing the flow rate increases the instability of the thermal conductivity detectors, and decreasing the flow rate increases the time required for an analysis. A very satisfactory compromise for general use is 50 ml. per minute. The instability of the detectors is not objectionable with flow rates below 100 ml. per minute. Reproduction of the flow rate to ±0.5% is necessary for quantitative work.

Adsorbents. The adsorbents used in this study included activated charcoal, silica gel, and alumina. Excellent separations were obtained on each of these materials. The choice of adsorbent depends largely on the composition of the mixture to be separated. Light hydrocarbons are readily separated on a column of silica gel or alumina at temperatures of 25° to 100° C. The order of elution from columns of these two adsorbents was generally the same, but, for the materials used, the retention volume of a given substance was greater on silica gel than alumina. Different results were obtained with adsorbents from different sources, but it was possible to get approximately the same behavior on adsorbents of the same kind from different sources by adjusting the temperature of the column. The order of elution of saturated hydrocarbons from charcoal was the same as from alumina or silica gel. Unsaturated hydrocarbons preceded saturated ones from carbon columns, but the reverse was true with the other two adsorbents. The most active charcoals were capable of good separations of hydrogen, oxygen, and methane, whereas the aluminas and silica gels investigated were very much poorer for the separation of these materials. Elution of the liquid hydrocarbons from charcoal required either a very long time or a temperature of 250° C. or above. Compounds such as octane and toluene were separated on alumina or silica gel in less than an hour at about 200° C.

Separations seem to be somewhat better on finer adsorbents, but this factor is not critical and there is little to be gained in using particles smaller than 50 mesh. The most satisfactory size seems to be about 30 to 50 mesh.

The adsorbents were sieved, washed with water to remove dust, and dried in an oven at 130° C. After being packed into the column, the carbon and silica gel adsorbents were dried further by passing the carrier gas through the column at a temperature of about 150° to 200° C. for several hours. The adsorptive properties of alumina were markedly dependent on its water content. The material used seemed to give the most efficient and reproducible separations when it was dried overnight by the carrier gas at the temperature at which it was to be used.

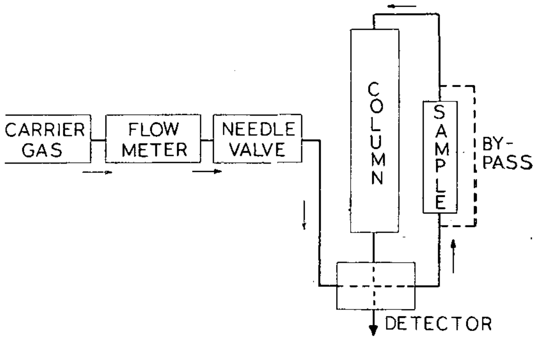


Figure 1. Schematic diagram of gas chromatography apparatus

The stability and reproducibility of any of the columns are improved by leaving them in operating condition continuously. If the carrier gas or column heater is turned off overnight, a warm-up period of 1 to 2 hours may be required to get the system in operation again. Unless the samples contain appreciable quantities of materials that are irreversibly adsorbed, the system can be used almost indefinitely without changing the adsorbent. For example, one silica gel column has been used for over 3 months for the routine determination of methane in a mixture of hydrocarbon gases without renewing the packing.

Columns. Glass columns with various dimensions were used. With small amounts of sample, the degree of separation seems to depend primarily on the length of the column. The amount of material that can be separated on a column of given length is roughly proportional to the cross-sectional area. The columns that were found most useful for general separations were about 0.5 cm. in diameter by 110 cm. long. All data included in this paper were obtained with columns having these dimensions, except where otherwise indicated.

The work reported here was done with columns at or above room temperature. Accurate thermostating is not necessary, except for the most careful work. It was found satisfactory in most cases to wrap the column closely with insulated Nichrome wire and then with asbestos tape. Electrical energy was supplied to the heater by means of a variable transformer. Continuously increasing the temperature of the column during a run makes possible a more efficient separation of materials having widely different affinities for the adsorbent. However, the experimental difficulties attending reproduction of heating rate and maintenance of constant flow rate eliminated the use of increasing temperature except for a few exploratory experiments.

Sample Containers. Liquid samples were applied directly to the end of the column by injecting them through a rubber serum cap with a hypodermic syringe. The usual volume of liquid sample was 0.01 to 0.1 ml. Gases can be introduced in the same way for qualitative results, if the syringe plunger is lubricated with a nonvolatile liquid in which the sample is relatively insoluble.

Glass sample containers constructed from two T-bore stopcocks were satisfactory for quantitative work with gases (see Figure 1). The sample was contained in one arm of the vessel, and the other arm was used as a bypass for the carrier gas until the system was ready for the sample. The volume of both arms was determined so that either could be used as the sample container. A sample volume of less than 15 ml. was generally used.

Detectors. Two general types of detectors were used. In the early stages of the work, the gases leaving the column were passed through a gas cell in an infrared spectrometer. This made qualitative and quantitative determination of the off gas possible at any time. Although this system was useful during the exploratory studies, the obvious disadvantages are its slow response due to the large volume of the gas cell and the necessity for adjusting the spectrometer to a different characteristic wave length for each component.

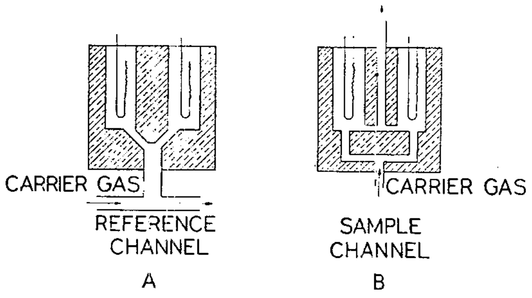


Figure 2. Thermal conductivity detector

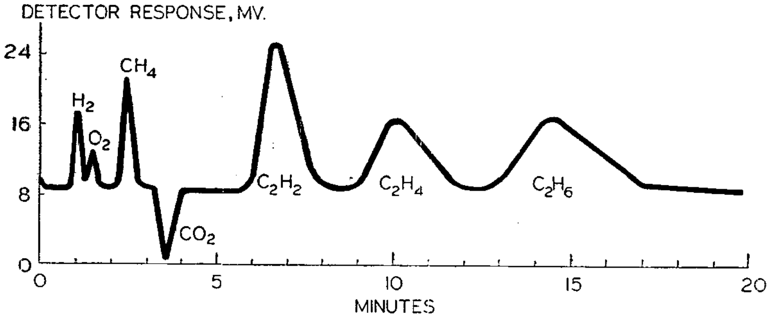


Figure 3. Elution of a mixture of gases from charcoal by nitrogen

The detectors used for most of the investigation were thermal conductivity cells. A cell similar to that described by Phillips (*15*) was found to have a low output signal, a response time longer than desirable, and objectionable sensitivity to ambient temperature changes. A commercially built cell (Gow-Mac Instrument Co., Madison, N. J.) was modified to pass the gases directly through two of the four filament chambers in order to reduce the response time. The result was a very satisfactory detector. It was considerably more sensitive to changes in flow rate than the unmodified cell, but the instability introduced by this factor was tolerable at the rates used.

These thermal conductivity cells were constructed so that the four filaments which were connected electrically to form the arms of a Wheatstone bridge were contained within a small, cubical, brass block. Before entering the column, the carrier gas was passed through the unmodified reference channel, where it came in contact with the two reference filaments (Figure 2,*a*). The gas leaving the chromatographic column was passed through the modified sample channel (Figure 2,*b*). Thus all four filaments were exposed to the carrier gas only, except when a sample component was being eluted. During elution of a component, two of the filaments (in opposite arms of the bridge) were exposed to the mixture of carrier gas and the component leaving the column. The resulting unbalanced voltage from the bridge was fed to a recording potentiometer which automatically plotted detector response against time.

The response of the thermal conductivity cell was affected very little by changes in ambient temperature. It was not necessary to provide a thermostat for the cell in locations where the variation in the surrounding temperature did not exceed ±5° C. For quantitative work, it was necessary to control the current through the cell to within ±1 ma.

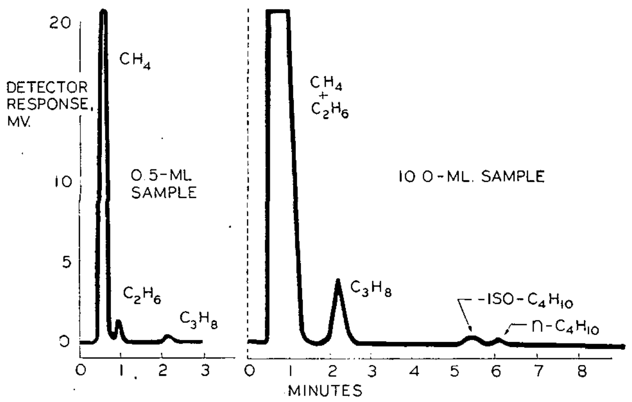


Figure 4. Elution of natural gas from alumina by hydrogen

The thermal conductivity detectors were used satisfactorily with recording potentiometers having ranges from 0 to 6 mv. to 0 to 30 mv. The stability of the background is believed to be sufficient to warrant use of a recorder having a range of 0 to 1 mv., if the ultimate sensitivity to minor components is important.

Thermal conductivity cells similar to those used in this study can now be obtained from the Gow-Mac Instrument Co.

DISCUSSION AND RESULTS

Separations. Gas chromatographic techniques involving elution from adsorbents seem to be most readily adaptable to the separation of relatively nonpolar substances boiling below 150° C. at atmospheric pressure. Except for closely similar isomers, hydrocarbons are usually separated with ease. During elution, most polar materials produce tailing, which leads to poor separation. Displacement development or the gas-liquid partition method can sometimes be used to obviate this difficulty (*3*, *12*).

The results presented illustrate typical separations. Figure 3 shows a curve for the elution of 10 ml. of a synthetic mixture of hydrogen, oxygen, methane, carbon dioxide, acetylene, ethylene, and ethane from a column of charcoal. The carrier gas was nitrogen, and the column temperature was about 180° C. The separation of natural gas on alumina at 60° C. with hydrogen as carrier

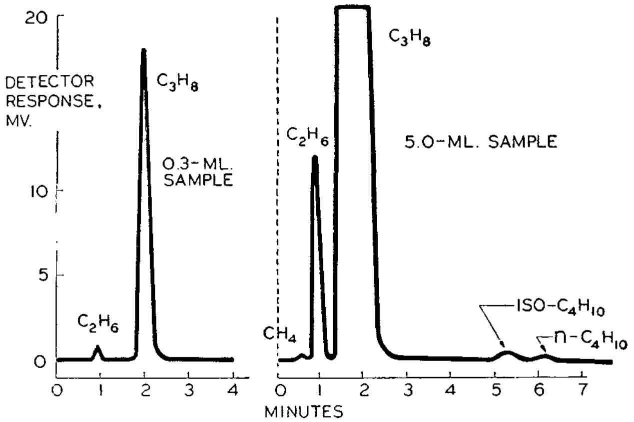


Figure 5. Elution of commercial propane from alumina by hydrogen

is illustrated in Figure 4. This same column and conditions were used for the separation of commercial propane (Figure 5), straight-run gasoline (Figures 6 and 7), and thermally cracked gasoline (Figures 8 and 9). The volume of the gasoline samples was 0.07 ml. Although there has not been sufficient investigation to establish the identity of the component responsible for each of the peaks produced by the gasoline samples, the presence of some compounds was easily established from the curves. The straight-run gasoline contained propane, isobutane, butane, at least two isomers of pentane, and at least three isomers of hexane. In addition to these, the sample of thermally cracked gasoline contained ethane, propylene, at least two isomers of butene, at least two isomers of pentene, and probably some hexenes.

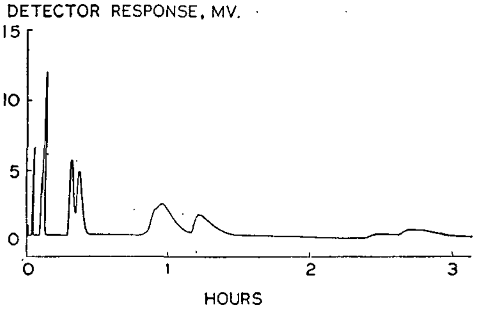


Figure 6. Elution of straight-run gasoline from alumina by hydrogen

The separation of a 2-ml. sample containing air, isobutane, butane, and *cis*- and *trans*-2-butene illustrates the efficiency of the method (Figure 10). The upper curve in the figure shows the separation obtained on a column of alumina 0.5 cm. in diameter by 110 cm. long at 60° C., with hydrogen as the carrier gas. The increased resolution obtained by using a similar column 220 cm. long is demonstrated by the lower curve.

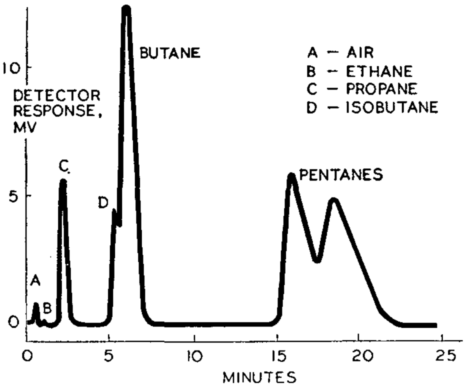


Figure 7. Elution of straight-run gasoline from alumina by hydrogen, with expanded time scale

Qualitative Identification. As in other elution techniques, the identification of a component can be based on its retention volume. As a constant flow rate was used, it was more convenient to measure the peak time—that is, the time elapsing between the introduction of the sample into the carrier stream and its appearance at maximum concentration in the detector. The corrected peak time is the measured value minus the time that would be required for an unadsorbed gas to pass through the same system. To a first approximation, the peak time of a given substance is independent of the amount of that component and of the presence of other materials. For a substance that tails during elution, the peak time decreases slightly as the quantity increases.

The logarithm of the corrected peak time is a linear function of the number of carbon atoms per molecule for saturated straight-chain hydrocarbons, as indicated by Figure 11. James and Martin (*11*) and Ray (*16*) found similar relationships between the logarithm of the retention volume and the number of carbon atoms for the elution of homologous series of several types of compounds from gas-liquid partition columns.

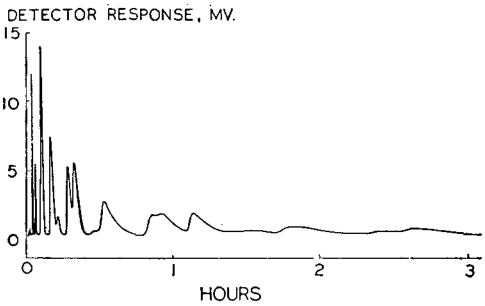


Figure 8. Elution of thermally cracked gasoline from alumina by hydrogen

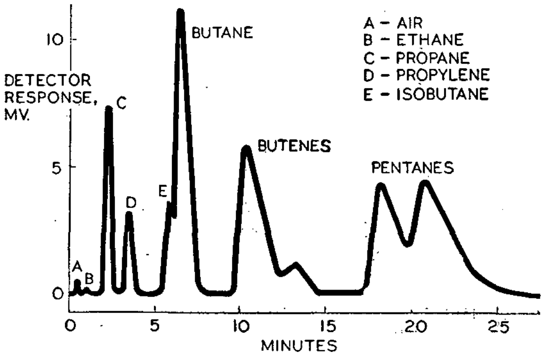


Figure 9. Elution of thermally cracked gasoline from alumina by hydrogen, with expanded time scale

Quantitative Determinations. Quantitatively, the height of the elution peak or the area under it is approximately proportional to the amount of the component that produced it. However, the relationship deviates from linearity for some gases, so that it is generally necessary to establish its shape by several experimental points. The peak height is influenced by the temperature of the column or any other factor that affects the activity of the adsorbent. It also depends somewhat on the volume of gases in

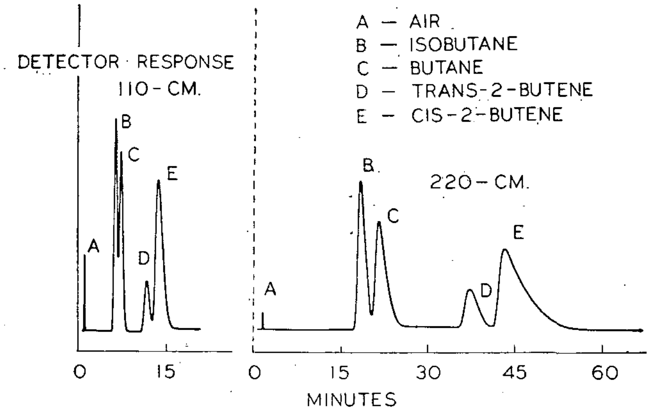


Figure 10. Effect of column length on separation efficiency

which the sample is contained. A given amount of a component produces a peak that decreases in height and increases in width as the total volume of sample increases. Therefore, if the peak height is employed for quantitative determinations, the vessels used for calibration must have the same volume as those containing the samples to be analyzed, or the results must be corrected for the change in peak height with sample size. This correction can be accomplished by making determinations with several volumes and extrapolating a plot of sample volume *vs.* percentage to the volume used for calibration.

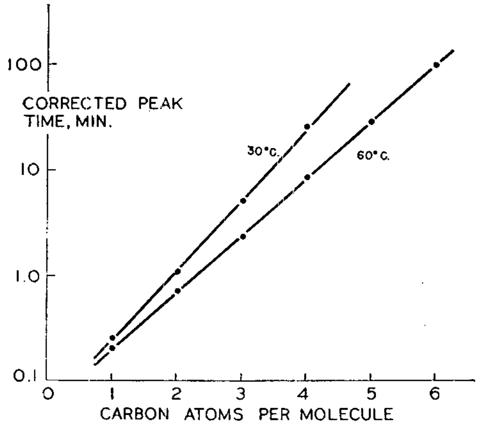


Figure 11. Peak times for elution of normal paraffins from alumina

Since the area under the elution peak is practically independent of the sample size, it is generally a more satisfactory quantitative measurement than the peak height. A polar planimeter is useful for this purpose, but it is simpler to use the product of the peak height and its half width (the width at one half the maximum height), as suggested by Cremer (*5*). There is no experimental evidence to indicate that determinations based on the peak height and half width are any less accurate than those obtained from planimetered areas.

The use of measured volumes of pure gases rather than mixtures greatly facilitates calibration. For this purpose, vessels similar to the sample containers but having much smaller volumes are used. Vessels having volumes that cover the desired concentration range are filled with the pure gas. The gas in each vessel is then put through the column. A plot of gas volume *vs.* response (area under the peak or peak height times half width) constitutes a calibration curve (Figure 12). A complete calibration usually requires less than an hour.

SUMMARY

Satisfactory gas chromatographic separations were obtained on charcoal, silica gel, and alumina columns using hydrogen, nitrogen, or carbon dioxide as carrier gases. A commercially built thermal conductivity cell was modified for faster response to produce an excellent detector.

In principle, the method is applicable to the qualitative and quantitative analysis of any mixture whose components can be separated by elution in the gaseous phase. It is already being used as a simple, accurate, and inexpensive means of analyzing hydrocarbon gases. It is estimated that hydrogen, methane, and ethane can be detected at a concentration of about 0.01%. The C_3 and C_4 hydrocarbons can be detected at a concentration of about 0.2%. Quantitative results are accurate to within about ±1 or 2% of the amount found.

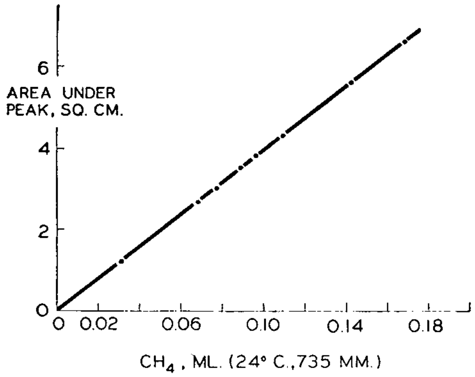


Figure 12. Typical calibration curve

In addition to the analytical applications based on the chromatographic elution curves, the method seems to have promise for the isolation of components for further study by other means. Concentration of minor components for identification by infrared absorption has already been accomplished. It seems likely that the combination of gas chromatographic separation and mass spectrographic identification would be worth while. Other detectors based on ultraviolet absorption or a surface potential device might be used to enhance the sensitivity to some substances.

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RECEIVED for review October 22, 1954. Accepted December 27, 1954.

Separation of Sulfur Compounds from Petroleum

C. J. THOMPSON, H. J. COLEMAN, H. T. RALL, and H. M. SMITH

Petroleum Experiment Station, Bureau of Mines, Bartlesville, Okla.

This paper reviews work on the newer methods of separation of sulfur compounds in petroleum, particularly as applied by American Petroleum Institute Research Project 48A. Included are the physical methods of distillation, adsorption, and thermal diffusion; and chemical methods, which include adduct and complex formation. Thermal diffusion, one of the more recently applied techniques, is stressed and its application to the separation of sulfur compounds and the constituents of crude oil is discussed. A list of 43 sulfur compounds (40 definite, 3 tentative) found in Wasson, Tex., crude oil is given. These were concentrated from the crude oil by one or more of the above methods and their semiquantitative identification was established by infrared absorption spectra or mass spectrometry. Sulfur compound types throughout the complete boiling range of a crude oil, as developed by adsorption and mass spectral data, are given.

THE American Petroleum Institute Research Project 48A has identified and semiquantitatively estimated the content of 43 sulfur compounds in distillates boiling to 220° C. from Wasson, Tex., crude oil. With the cooperation of the Humble Oil and Refining Co. it has been shown that, in crude oil topped to 150° C. and deasphaltened, there are at least 11% of sulfur compounds and that 65% of these compounds are benzothiophenes. As these benzothiophenes are for the most part high boiling, this means that at least 20% and perhaps much more of the higher boiling parts of Wasson crude oil are polycyclic benzothiophenes.

These advances have been made possible because of the newer separative and mass spectrometer techniques available in recent years. Distillation, thermal diffusion, and especially adsorption on alumina are the tools used to accomplish most of the basic separations. Chemical methods play a smaller but important part in certain phases of the work. This paper presents the background for the concentration of sulfur compounds as conducted by the project to July 1, 1954.

PHYSICAL METHODS OF SEPARATION

Many sulfur compounds are thermally unstable compared to hydrocarbons; therefore distillation, the usual tool for effecting fractionation of crude oil, must be used with caution. Contact with metals, especially reactive metals such as copper and mercury, must be eliminated or minimized. For these reasons other methods of separation, such as adsorption and thermal diffusion, have been preferred for most of the work of the American Petroleum Institute project. Distillation has been used only to obtain a primary distillate, for simple fractionation of this distillate, and for final fractionation of a sulfur compound concentrate. To minimize decomposition, all of these distillations are conducted under carefully controlled conditions of temperature, heating time, and metal contact.

DISTILLATION

Preparation of Primary Distillate. An all-glass isothermal still was developed to meet the thermal and contact time restrictions that were necessary in processing the crude oil.

This stripping unit is operated at atmospheric pressure and at a temperature of 100° C., with feed rates of approximately 2 liters of crude oil per hour. The crude oil is delivered by a gear pump to the top of a glass drum rotating at about 170 r.p.m. and heated internally by steam. As the oil flows down the rotating drum, it is swept by a countercurrent stream of inert gas, and the vapors, flashed from the thin film of heated oil, are carried by the inert gas into a partial condenser. The amount of reflux can be controlled by the temperature of the coolant used in this condenser. For maximum stripping a partial condenser is not employed and the vapors pass directly from the isothermal still into the collection system. The vapors that are not condensed in receivers and cold trap are led into a cooled liquid absorber for final scrubbing. The degree of stripping is controlled by variations in crude oil feed rate, by the flow rate of the countercurrent stream of inert gas, and by the occasional use of a partial condenser. Distillates with an end point of 240° C. have been prepared with this equipment. Such processing can remove from the crude oil essentially all the distillate boiling below 150° C., but as the end points of distillates increase beyond this point increasing quantities of the higher boiling compounds remain in the residuum.

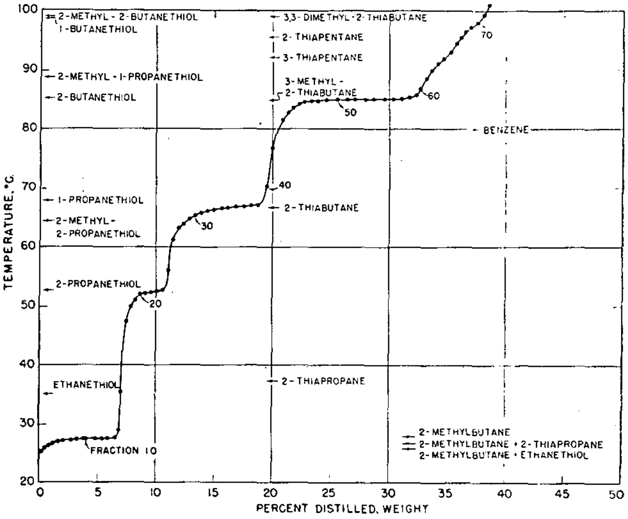

Figure 1. Fractionation of sulfur concentrate from Wasson distillate

Because the temperature never exceeds 100° C. and the contact time is less than 30 seconds, thermally labile compounds have little opportunity to decompose. Under such conditions of time and temperature, distillates have been prepared that show no evidence of cracking of either hydrocarbons or sulfur compounds. The residue from this stripping operation is retained for subsequent work in the higher boiling ranges.

Simple Fractionation. A 30-plate glass Oldershaw column is used to separate the distillate obtained from the isothermal still into fractions of suitable boiling range before the sulfur compounds are concentrated by adsorption. In these distillations pot temperatures are kept below 100° C. to prevent possible decomposition or other changes in the sulfur compounds. Such distillations are conducted at reduced pressures when necessary, and no evidence of decomposition has been noted.

Microfractionation. Concentrates of the sulfur compounds obtained by adsorption and chemical treatment are carefully fractionated before identification. Two columns are employed in this work, an 8 × 300 mm. Heligrid-packed column and a 1000-mm. all-glass concentric tube column. With both columns, efficient fractionation of small quantities of material, in some cases less than 5 ml., has been accomplished. Figure 1 illustrates a distillation conducted in the Heligrid-packed column. The distillation curve shows distinct plateaus for the sulfur compounds present in the concentrate. The names and boiling points of the theoretically possible sulfur compounds are given at the margins.

ADSORPTION

Silica Gel. The chromatographic method of adsorption, as introduced by Day (*4*) in 1900, has been modified through the years for application to components of petroleum. Two general adsorption techniques have been developed—the displacement method (*1, 7, 25, 26*) and the elution method (*11, 20, 24*). The adsorption technique for concentrating the sulfur compounds present in straight-run petroleum distillates (containing 0.1 to 0.5 weight % sulfur) utilizes elution followed by complete displacement.

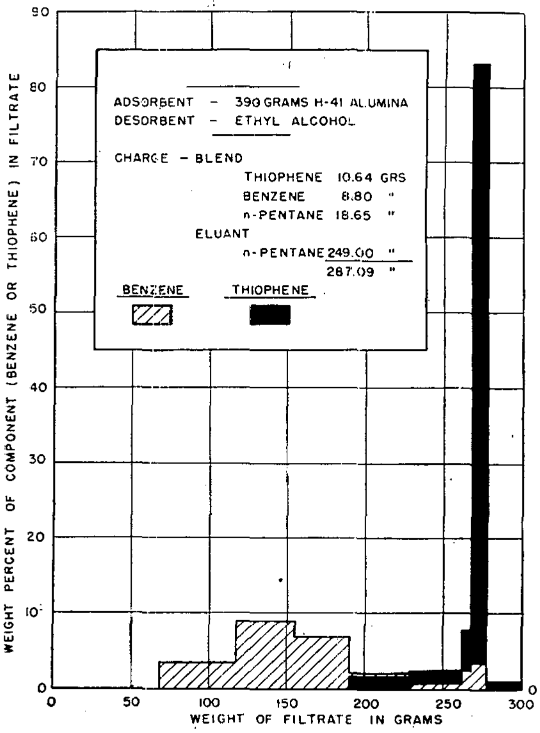

Figure 2. Separation of benzene and thiophene by alumina adsorption

Silica gel, as an adsorbent for various sulfur compounds, was investigated as early as 1926 (*29, 30*). Nineteen years later, Mair (*23*), in developing a silica gel adsorption analysis for petroleum distillates, found that sulfur compounds present in the distillate interfered with the aromatic analysis and thus required the use of a small correction. Dineen *et al.* (*6*) later pointed out that the properties of an aromatic fraction obtained by silica gel adsorption of high-sulfur shale-oil naphthas are considerably modified by the presence of sulfur compounds. They conducted some adsorption experiments with silica gel using blends of different sulfur compounds in toluene and found that some sulfur compounds were not completely separable from the toluene.

Haresnape, Fidler, and Lowry (*8*) in their paper on the separation of sulfur compounds by adsorption on silica gel, in addition to indicating the relative adsorptive strengths of sulfur compound types, also showed that some sulfur compounds were not completely separable from toluene. Preliminary experimental work conducted in this laboratory on a petroleum distillate confirmed these findings, showing that, although a majority of the sulfur compounds present in the distillate tend to concentrate at the trailing edge of the aromatic fraction, a significant quantity of sulfur compounds occur throughout the entire fraction, so as to require inclusion of all the aromatics in the "sulfur concentrate." This is not desirable, because inclusion of substantial quantities of aromatics with the sulfur compounds seriously hampers separation and identification of the individual sulfur compounds in subsequent procedures. Attention was therefore turned to alumina as an adsorbent.

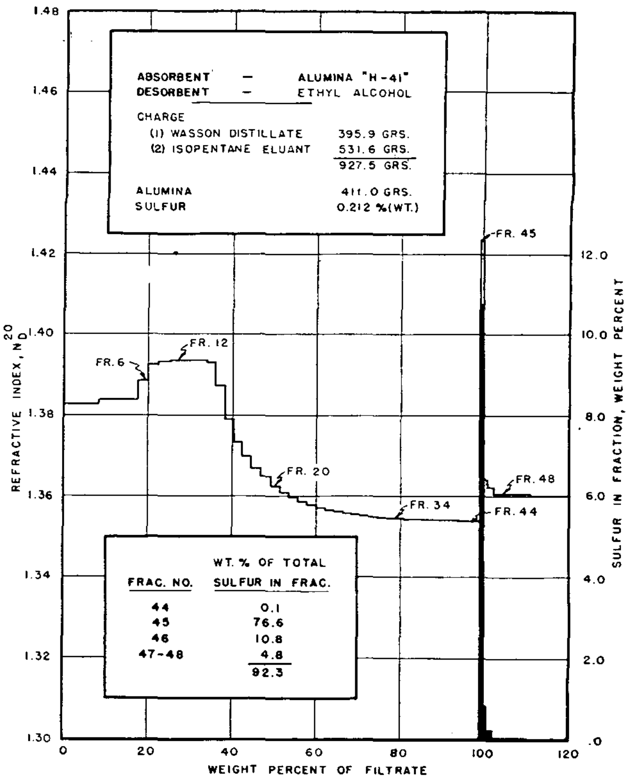

Figure 3. Alumina adsorptogram of Wasson distillate

Alumina. Application to Distillates. Extensive experimentation showed that H-41 activated alumina (Aluminum Co. of America) is effective in separating sulfur compounds from aromatics. This is evident in Figure 2, which illustrates the separation obtained between benzene and thiophene, two compounds that are very closely related in boiling point and adsorption strength on silica gel (*6, 8*).

Three distillates having boiling ranges of 38° to 111°, 111° to 150°, and 150° to 220° C. have been tested to date. The technique used for concentrating the sulfur compounds present in these is essentially the same. The distillate is percolated through the alumina, eluted by an appropriate paraffin hydrocarbon (isopentane, pentane, etc.), until the refractive index of the eluate approaches that of the eluant, and then ethyl alcohol is added to displace the remaining sulfur compounds completely. The weight ratio of sample to gel can be as high as 1 to 1, as was used in a typical experimental run shown in Figure 3, but it is usually about 1 to 2 to minimize wash-through of sulfur compounds with the eluant. The adsorptogram given in Figure 3 shows that paraffins and naphthenes are first eluted, followed by the aromatics that are being selectively displaced from the sulfur compounds by elution. The peak in the adsorptogram, marked Fr. 45, is the fraction of highest sulfur-compound concentration, about 11 weight % sulfur. Following this fraction are two or three alcohol fractions rich enough in sulfur compounds to warrant recovery by diluting with isopentane and washing out the ethyl alcohol with salt water.

A single-pass filtration will not remove all aromatics from the sulfur compound concentrate, but two or three reruns of this sulfur compound concentrate combined with the isopentane extracts from the alcohol fraction will reduce the aromatic content to a negligible value. The resulting sulfur compound concentrate is then ready for chemical treatment and distillation as required.

Application to Crude Oil. Most separations utilizing adsorption techniques deal with samples of limited boiling range, such as gasoline, kerosine, gas oil, or asphalts. However, because high temperatures are avoided, adsorption separations based on the whole crude oil are advantageous for studying sulfur compounds that occur naturally in crude oil. Adsorption was applied to total crude oils as early as 1897 (*5*) utilizing, primarily, straight percolation. The application of elution techniques to aid fractionation of crude oils appeared in 1944 (*27*), and a more complete analytical treatment was published in 1954 (*17*). Early (1952) experimental work by the American Petroleum Institute on the direct chromatography of crude oils indicated that for characterization with respect to sulfur compounds present, the crude-oil sample could be advantageously modified by topping to 150° C. and removing asphaltenes with pentane.

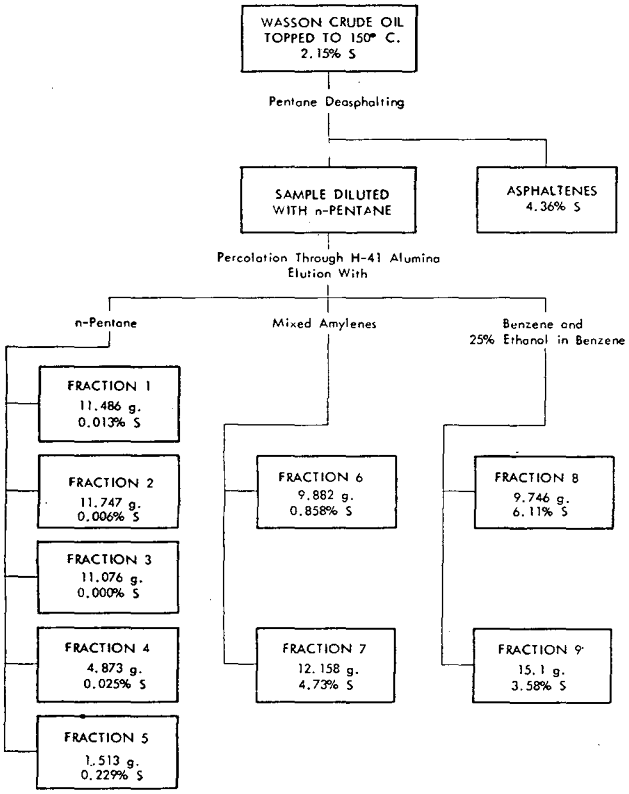

Figure 4. Preparation of Wasson crude oil fractions for identification of sulfur type

A sample of Wasson residue from the stripping operation conducted in the isothermal still was combined with an appropriate quantity of Wasson distillate (150° to 220° C.) to yield a sample representative of Wasson crude oil topped to 150° C. The asphaltenes were removed from this topped sample by diluting 1 volume of the topped crude oil with 7 volumes of *n*-pentane, storing in a cold room (38° F.) for precipitation and settling of asphaltenes, and filtering. The filtrate, representing deasphaltened Wasson crude oil topped to 150° C. and diluted with 7 volumes of *n*-pentane, was used directly for adsorption analysis.

A portion of this filtrate containing 84 grams of topped and deasphaltened Wasson crude oil was percolated through an adsorption column containing activated H-41 alumina (Alcoa spray-dried, fluid grade) and eluted with solvents of varying adsorptive strengths.

The adsorption column utilized for this analysis was a glass tube, 2 cm. in inside diameter and 225 cm. long, having spherical joints (35/20) at both ends to allow attachment of a reservoir at the top and a stopcock at the bottom and to facilitate cleaning. A sample-to-gel weight ratio of 1 to 7 was used in these experiments.

The various classes of hydrocarbons and sulfur compounds are selectively displaced by using a weakly adsorbed initial developer, followed by other eluants possessing greater adsorptive strengths (*19 28*). The selective displacement of sample from alumina was accomplished by eluting successively with 1000 ml. of *n*-pentane, 1500 ml. of mixed amylenes, 250 ml. of benzene, and 1200 ml. of an ethyl alcohol–benzene mixture (25% ethyl alcohol). Possibly the analysis could have been improved somewhat by inclusion of cyclohexene as an additional incremental eluant between the amylenes and benzene. The solvents used, besides giving excellent class separations, gave high quantitative yields, in that the recoveries were slightly above 100% with respect to both sample and sulfur compound charge (105 and 103%, respectively).

The eluate from the bottom of the column passed directly into

Table I. Results of Mass Spectral Study of Alumina Adsorption Fractions of Topped-Deasphaltened-Wasson Crude Oil

Eluants	*n*-Pentane					Amylenes		Benzene and Benzene–Ethyl Alcohol	
Fraction No.	1	2	3	4	5	6	7	8	9
Color	Colorless	Colorless	Colorless	Colorless	Colorless	Very light straw	Yellow	Reddish brown	Blackish brown
Refractive index	1.4442	1.4500	1.4514	1.4567	1.4799	1.5060	1.5399	1.595	Too dark
Charge (output basis)[a], wt. %	13.1	13.4	12.6	5.6	1.7	11.3	13.9	11.2	17.2
Compound type, wt. %									
Hydrocarbons									
Saturates	100.0	100.0	100.0	100.0	75.7	0.0	0.0	0.0	...
Aromatics									
1 Ring	0.0	0.0	0.0	0.0	22.0[b]	94.4	26.4	0.0	...
2 Rings	0.0	0.0	0.0	0.0	0.0	0.0	23.2	9.8	...
3 Rings	0.0	0.0	0.0	0.0	0.0	0.0	11.2	23.4	...
4+ Rings	0.0	0.0	0.0	0.0	0.0	0.0	0.0	3.5	...
Sulfur compounds									
Thiophenes									
1 Ring	0.0	0.0	0.0	0.0	2.3[b]	3.2	0.0	0.0	...
2 Rings[c]	0.0	0.0	0.0	0.0	0.0	0.0	22.3	4.7	...
3 Rings[d]	0.0	0.0	0.0	0.0	0.0	0.0	2.5	29.0	...
4+ Rings	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	...
Sulfides[e]	0.0	0.0	0.0	0.0	0.0	2.4	14.4	9.6	...
Other S compounds[f]	...	...	...	...	...	...	...	...	...
Oxygen + nitrogen compounds	...	...	...	...	...	...	...	20.0	...
Total	100.0	100.0	100.0	100.0	100.0	100.0	100.0	100.0	
S calcd.[g], wt. %	0.000	0.000	0.000	0.000	0.29	0.72	5.01	5.55	...
S obsd., wt. %	0.013	0.006	Nil	0.025	0.229	0.858	4.73	6.11	3.58

[a] Charge, 76.1% of original crude oil.
[b] Estimated from ultraviolet.
[c] Benzothiophenes.
[d] Dibenzothiophenes or thiophenonaphthalenes.
[e] Iodine complex method (*9*).
[f] Free S, mercaptans, disulfides, and polysulfides below limits of detectability of polarograph.
[g] Average molecular weight estimated from mass spectra to be about 250.

a continuous stripper (*12*) which provided samples free of eluant. The stripper was heated by boiling isopropyl alcohol (boiling point 82.5° C.) when the eluants were *n*-pentane or mixed amylenes, and by methyl Cellosolve (boiling point 124° C.) for the eluants benzene and ethyl alcohol. When the eluate became black (ethyl alcohol–benzene eluant), the stripper was removed, and the entire remaining eluate was collected as one sample. This was later vacuum distilled and further treated by evaporation in a heated vacuum desiccator for removal of benzene and alcohol.

The entire sample treatment is shown diagrammatically by Figure 4, and characterization of the fractions from the above treatment is presented in Table I. The mass spectrometer analysis of these fractions was obtained by the Humble Oil and Refining Co., employing its high resolution mass spectrometer. Details of the qualitative and quantitative methods of analysis, as developed in the Humble Laboratories and applied to these fractions, are given by Hastings, Johnson, and Lumpkin (*10*). The data presented in Table I indicate that *n*-pentane was very selective in separating saturates from aromatics. Mixed amylenes have been used previously in this laboratory for separating cyclic aromatics from dicyclic aromatics (*22*). This particular adsorption analysis shows a comparatively sharp break in composition occurring between fractions 6 and 7. If the material in fraction 7 had been collected as two smaller fractions, the break would have been even more pronounced.

Mixed amylenes appear unable to displace many three-ring aromatics or thiophenes and fail to displace any measurable amounts of four-ring compounds. It is believed that, if cyclohexene had been used following the amylenes and before benzene, an even better separation could have been obtained between the two- and three-ring compounds, as well as between the three and higher numbered ring compounds.

These data are the basis for several interesting observations: Neglecting the composition of fraction 9, on which few analytical data are available, sulfur compounds represent 11% of the crude oil boiling above 150° C.; almost 70% of these sulfur compounds are thiophenes; and of these thiophenes the proportions of those having one, two, and three rings are 1, 9, and 9, respectively. Fraction 9 was an extremely heavy, tarlike material, unsuitable for mass spectrometer analysis. Information on the composition of this fraction would undoubtedly shift the above proportions in the direction of more three and higher ringed thiophenes and increase the total sulfur compounds to more than 15% of topped Wasson crude oil. Chromatography over alumina is very effective in separating the several thiophenes according to the number of aromatic rings condensed with the thiophene ring; chromatography of a crude oil topped to 150° C. and deasphaltened seems to offer an efficient and practicable method of effecting separation of sulfur compounds under conditions where no decomposition should occur.

THERMAL DIFFUSION

Application to Distillates. An example of the data obtained from thermal diffusion of a complex mixture is indicated in Figure 5, where refractive indices of the thermal diffusion fractions are plotted. These data, with sulfur contents of selected fractions, show that a significant separation or concentration of sulfur compounds was obtained by applying liquid thermal diffusion to this Wasson crude oil distillate having a boiling range 150° to 220° C. The sulfur content of fraction 10 was 150 times that of fraction 1.

A concentrate of chain and cyclic sulfides and aromatic hydrocarbons, with a sulfur content of 7.4 weight %, was prepared from the above distillate and subjected to thermal diffusion. Although some concentration of sulfur compounds was effected (fraction 1, 6.2%; fraction 10, 13.1%), the high aromatic content, coupled with the mixture of many chain and cyclic sulfides, precluded the concentration of all the sulfur compounds in the lower end of the column. However, one of the principal objectives of this experiment was to see whether the chain and cyclic sulfides could be separated by thermal diffusion.

Table II. Data from Mass Spectral Study of Thermal Diffusion Fractions from Sulfide-Aromatic Concentrate

Component	Concentration, Vol. %	
	Fraction 1	Fraction 10
Alkyl benzenes	64	15
Naphthalenes	1	10
Chain sulfides	20	0
Monocyclic sulfides[a]	14	65
Dicyclic sulfides[a]	1	10
	100	100

[a] General formulas $C_nH_{2n}S$ and $C_nH_{2n-2}S$, respectively. Sulfur not necessarily in ring.

Limited mass spectrometer data, as shown in Table II, indicate that a fair degree of segregation was realized between types of compounds. Removal of the aromatics before the application of thermal diffusion should materially increase the resolution of the mixture and the accuracy of the analysis.

Application to Crude Oil. A sample of topped Wasson crude oil was subjected to liquid thermal diffusion. The material utilized in this experiment was prepared as described above by the addition of an appropriate amount of distillate (boiling above 150° C.) to a sample of residue from the isothermal still, resulting in a topped crude oil with an initial boiling point of 150° C. The asphaltenes were not removed from this sample, as contrasted with the topped deasphaltened sample used in the adsorption studies previously described.

This material was placed in a thermal diffusion column for 168 hours. The ten fractions collected at the end of the experiment were markedly different in color, viscosity, and sulfur content. Some properties of the fractions are given in Table III. The data currently available, while admittedly limited, suggest that a remarkable separation was achieved, and further characterization of these fractions by mass spectrometer analysis should aid in the evaluation of the usefulness of liquid thermal diffusion when applied to crude oils.

CHEMICAL METHODS OF SEPARATION

Chemical methods have been investigated, as a means of not only separating sulfur compounds from petroleum or its fractions, but also of separating different types of sulfur compounds by

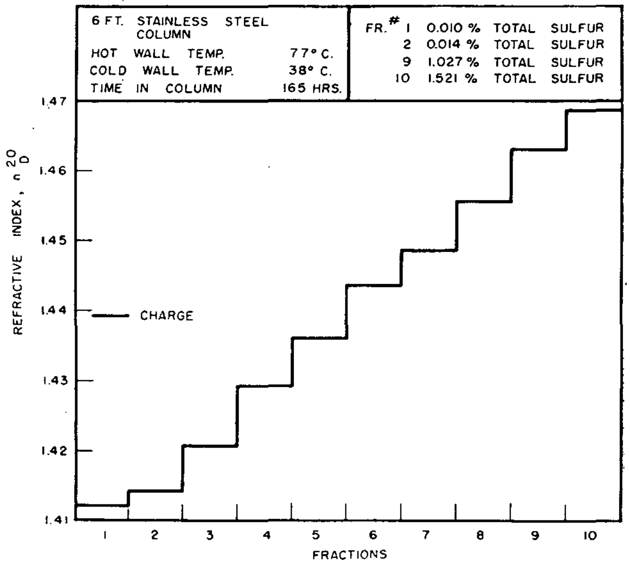

Figure 5. Fractionation of Wasson distillate boiling above 150° C. by liquid thermal diffusion

utilizing the chemical reactivity of the compounds. In the earlier work in this field, several methods were reported in the literature for chemical separation of thiols. Some of these methods are of questionable value because of possible chemical alteration of materials being investigated. Other methods are applicable only to limited extent—for example, treatment with sodium hydroxide solutions for thiol sulfur removal is quantitative only in the lower molecular weight range. Zandona and Rippie (*31*) report that in pilot plant experiments only 2% of C_7 thiols were removed with 10% caustic and only 73% were removed with 40% caustic. Methods of extraction must be as nearly quantitative as possible and produce little change in structure, if identification and quantitative determination of each sulfur compound are to be realized.

Table III. Properties of Fractions from Thermal Diffusion of Wasson Crude Oil

Fraction No.	Sulfur, Wt. %	n_D^{20}	Color
1	0.07	1.4296	Water white
2	0.17	1.4412	White waxy semisolid
3	0.52	1.4517	Very light yellow
4	0.87	1.4666	Light yellow
5	1.47	1.4823	Yellowish brown
6	1.98		Dark reddish brown
7	2.86		Black
8	3.33		Black
9	3.53		Black
10	3.60		Black

Recently Hopkins and Smith (*13*) described a method for the extraction and recovery of thiols from petroleum distillates. This method is used in this laboratory in conjunction with the other separation processes. In applying the method, a concentrate of sulfur compounds containing thiols is diluted with isopentane and made to react with 2*N* sodium aminoethoxide ($H_2NC_2H_4ONa$) dissolved in anhydrous ethylenediamine. The thiols are regenerated from this solution by acid hydrolysis and recovered by steam distillation. The data indicate that the procedure is essentially quantitative. Distillates can be treated in this manner, but it is preferable to concentrate the sulfur compounds by adsorption on alumina before extraction to avoid handling excessive quantities of material. This method has been used successfully on distillates boiling to 220° C.

The separation of cyclic and chain sulfides by chemical methods has been studied. Cyclic sulfides are more reactive chemically toward such reagents as mercury salts, salts of certain other heavy metals, and acids than are the chain sulfides. Birch and McAllan (*2*) have published a method of separating cyclic and chain sulfides based on their differential reactivity with mercuric salts. This method has two disadvantages, in that several extractions must be made, which result in a series of fractions of decreasing cyclic content, and compounds having a tertiary carbon-sulfur linkage are largely destroyed.

Other reagents have been investigated for making this separation. Zinc chloride was found to be more selective than mercuric salts but less reactive, and with this reagent no destructive action on tertiary C-S compound has been observed. However, separation of chain and cyclic sulfides by chemical means is somewhat limited because of the small differences in chemical reactivity between the two types of sulfides. Multiple extractions with various concentrations of zinc chloride are required, and some loss occurs with each extraction. New methods must be developed or old ones refined to place chemical methods of separation of sulfides in competition with separation by adsorption and thermal diffusion.

In the ultimate characterization of mixtures of sulfur compounds, the separation of primary and secondary thiols from tertiary thiols would be desirable. With certain limitations and exceptions the formation of insoluble ammonium mercaptides of the primary and secondary thiols accomplishes this objective. Primary thiols readily form ammonium mercaptides with liquid ammonia. With the exception of those compounds containing an aromatic ring, the mercaptides of six or more carbon atoms are sparingly soluble and dissociate upon removal of excess ammonia and warming to room temperature. Tertiary thiols of this molecular weight range do not form ammonium mercaptides to an appreciable extent and any mercaptide formed remains in solution. All thiols below C_6 form mercaptides of moderate solubility with little apparent difference in reactivity of primary and tertiary thiols. Few secondary thiols have been tested, but the limited data suggest that secondary thiols, at least in the C_6 to C_{11} range, will react and can be recovered with the primary thiols, leaving the tertiary compounds unreacted. The method is applicable only to the C_6 to C_{12} range.

Urea and thiourea have been found useful in some cases in separating straight- and branched-chain compounds.

DEVELOPMENT OF NEW TECHNIQUES

Thermal Diffusion. The phenomenon of thermal diffusion was first reported by Ludwig (*21*) in 1856. From that date until about 1948, literature references to the process were scarce and dealt in many cases with the application of gaseous thermal diffusion. In recent years liquid thermal diffusion has attracted the attention of several investigators. In 1951 Jones (*14*) developed a continuous process of liquid thermal diffusion for the separation of various petroleum stocks. Additional papers by Jones (*15, 16*) report the study of tall oil in glass thermal diffusion columns and research on the separation of organic liquid mixtures.

The exact mechanism of liquid thermal diffusion is not known. However, the direction and magnitude of the concentration or separation are believed to depend to a great extent upon the relative shapes or configuration of the molecules present in the mixture. Separations can be made of compounds having identical molecular weights and boiling points. Many isomers may be efficiently separated. It has been observed that certain molecules, such as straight-chain molecules, will migrate to the hot wall and rise, while more compact ring structures will concentrate at the cold wall and settle.

For the separation of liquid hydrocarbon mixtures, Kramers (*18*) proposed the following qualitative rules:

1. In general, in the case of mixtures of substances with the same boiling point, the component with the smallest molecular volume will tend to move toward the cold wall.
2. In mixtures of substances with about equal molecular volumes and boiling points, the component with the smallest surface area of its molecules tends to move toward the cold wall.
3. In mixtures of molecules that have equal molecular volumes and equal surface areas, the higher boiling component tends to move toward the cold wall.
4. Of a homologous series, the component with the largest number of carbon atoms tends to move toward the cold wall.

The literature is devoid of experimental data on the employment of thermal diffusion in the separation of sulfur compounds. The principal reason for this has been the unavailability of sulfur compounds in sufficient quantity and of good purity. The process of liquid thermal diffusion has received attention, in this laboratory, as a possible tool to augment other separation processes in the over-all program of concentration and separation of sulfur compounds. This work has included synthetic mixtures of hydrocarbons, hydrocarbons and sulfur compounds, and various types of sulfur compounds.

Application to Pure Sulfur Compounds. Most sulfur compounds used in this study were either commercially available samples or second-purity material from the preparation of high-purity API-USBM standard samples. Fair agreement was observed between the determined and literature values of the index of refraction. Unless otherwise stated, all binary mixtures studied were 50-50 volume % of each component. The index of refraction of each fraction was used as a measure of the separation attained, and the composition of selected fractions was determined by infrared or mass spectra.

Table IV. Separation of Binary Mixtures of Sulfides by Thermal Diffusion

Components	Vol. %	B.P., °C.	Density, 20° C.	Mol. Wt.	Mol. Vol.	n_D^{20} Detd.	n_D^{20} API	Time in Column, Hours	Composition, Vol. % Fr. 1	Fr. 2	Fr. 9	Fr. 10	Degree of Separation[a]
4-Thiaheptane	50	142.83	0.8377	118	140.9	1.44916	1.4487	...	98.9	99.1	...	...	
Thiacyclohexane	50	141.75	0.9856	102	103.5	1.5064	1.5067	114	...	...	99.4	98.9	Excellent
2,8-Dimethyl-5-thianonane	50	216	0.833	174	208.9	1.45288	...	...	98.7	97.4	...	...	
Thiacyclohexane	50	141.75	0.9856	102	103.5	1.50584	1.5067	170	...	...	98.1	99.1	Excellent
3,5-Dimethyl-4-thiaheptane	50	165	0.835	146	174.8	1.45048	...	...	99.3	99.2	...	...	
Thiacyclohexane	50	141.75	0.9856	102	103.5	1.50555	1.5067	185	...	0.8	97.5	98.4	Excellent
6-Thiaundecane	50	229	0.839	174	207.4	1.45641	...	...	50	...	...	...	
2-8-Dimethyl-5-thianonane	50	216	0.833	174	208.9	1.45234	...	250	50	...	...	...	No separation
6-Thiaundecane	50	229	0.839	174	207.4	1.45682	...	...	100	...	...	...	
(1-Thiahexyl)cyclopentane	50	...	...	172	...	1.48046	...	210	...	...	...	95	Good
4-Thiaheptane	50	142.83	0.8377	118	140.9	1.44914	1.4487	...	57.1	56.7	...	...	
2-Thiaheptane	50	145	0.8431	118	140.0	1.45106	1.4506	167	...	...	63.9	69.6	Fair

[a] Based not only on data reported in columns 10 to 13 but also on nature of refractive index-volume per cent curves and other factors.

This work was conducted in 6-foot stainless steel columns of the concentric tube type. The columns have an annulus of 0.012 inch, a mean annulus diameter of 0.635 inch, and a sample capacity of 30 ml. The inner concentric tube is cooled with tap water flowing from bottom to top, and the outer tube is heated electrically with a resistance winding. Three thermocouples are silver-soldered directly to the hot wall at the top, center, and bottom of the column; thermocouples are also attached to the water inlet and outlet lines. The reported hot and cold wall temperatures are an average of the thermocouple readings taken during the experiment. In addition to the temperature gradient existing between the hot and cold wall, there exists a vertical temperature gradient within the column. The hot wall temperature should not exceed the boiling or cracking temperature of the material being processed, and with that limitation in mind the column should be operated at as high a temperature level and with as great a temperature gradient as feasible.

The columns employed were designed for, and have been used only in, batchwise experimentation. Each column has 11 ports spaced about 7 inches apart along the length of the outer tube for the removal of 10 samples from the annular space. The sample is introduced through the center port and permitted to flow by gravity into the column from an overhead reservoir. The feed line may or may not be closed during operation; but for the work reported here, it was left open until immediately before the run was completed and the fractions were removed. The sample is left in the column for the desired number of hours (48 to 200), then the feed line is closed and 10 fractions of about 2.6 ml. each are taken in order from top to bottom.

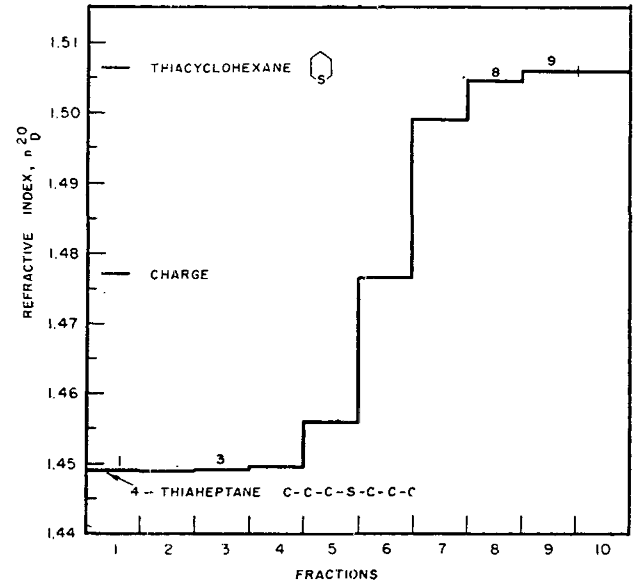

Figure 6. Separation of thiacyclohexane and 4-thiaheptane by liquid thermal diffusion

6-foot stainless steel column
Hot wall temp., 72° C.
Cold wall temp., 16° C.
Time in column, 114 hours

Fraction No.	Constituent	%
1	4-Thiaheptane	98.9
3	4-Thiaheptane	99.1
8	Thiacyclohexane	96.6
9	Thiacyclohexane	99.4

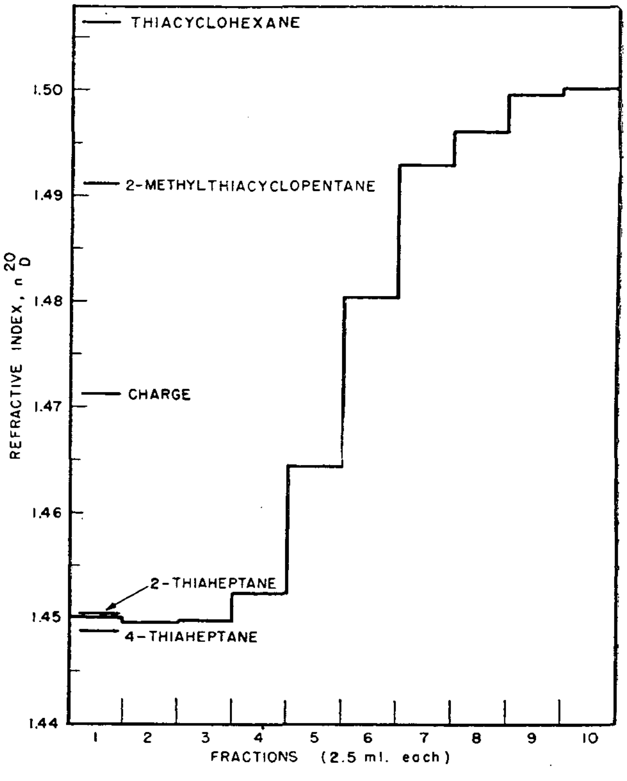

Figure 7. Separation of open-chain and cyclic sulfides by liquid thermal diffusion

6-foot stainless steel column
Hot wall temp., 69° C.
Cold wall temp., 31° C.
Time in column, 154 hours

Fraction No.	Constituent	%
1	Cyclic sulfides	<0.5
2	Cyclic sulfides	<1
3	Cyclic sulfides	<1
4	Cyclic sulfides	2-3
7	Chain sulfides	2
8	Chain sulfides	<1
9	Chain sulfides	<1
10	Chain sulfides	<0.5

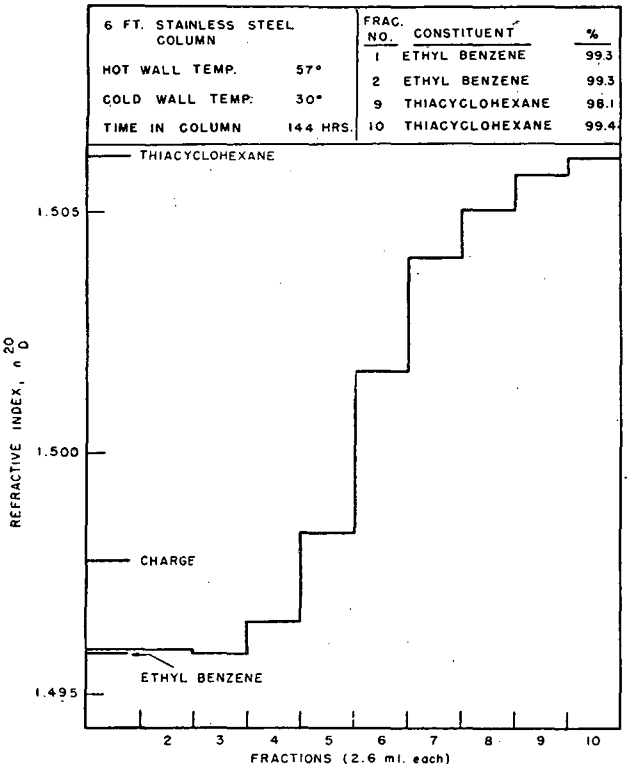

Figure 8. Separation of ethylbenzene and thiacyclohexane by liquid thermal diffusion

Figure 6 is a typical example of the type and degree of separation that may be achieved by means of thermal diffusion. The boiling points of thiacyclohexane and 4-thiaheptane are 141.8° and 142.8° C., respectively. The separation of these two sulfides by ordinary distillation would be difficult. Certain limitations also exist in the separation of this or similar mixtures by either routine absorption techniques or chemical treatment.

The excellent degree of separation achieved with thiacyclohexane and 4-thiaheptane is not limited to binary mixtures. Figure 7 illustrates equally good separation with a mixture of two chain sulfides and two cyclic sulfides. Excellent separations are also realized with 50-50 volume mixtures of thiacyclohexane and 2,8-dimethyl-5-thianonane (isoamyl sulfide), and thiacyclohexane and 3,5-dimethyl-4-thiaheptane (*sec*-butyl sulfide).

Table IV gives the data for the separation of several binary mixtures of sulfides. Mixtures of components with as similar a structure as 4-thiaheptane and 2-thiaheptane, and (1-thiahexyl) cyclopentane (amyl cyclopentyl sulfide) and 6-thiaundecane (amyl sulfide) can be resolved by liquid thermal diffusion.

Cyclic sulfides and aromatics can be separated with excellent results, if thiacyclohexane and ethylbenzene can be considered representative of these two classes of compounds (see Figure 8). A chain sulfide (4-thiaheptane) and ethylbenzene show very little tendency to separate by this process. A previous reference to thermal diffusion of a sulfide concentrate containing aromatics showed this same difficulty.

Table V shows data resulting from the separation of several mixtures of sulfur compounds, and sulfur compounds and hydrocarbons. The molecular volume, as reported in the tables, was calculated by dividing molecular weight by density at 20° C. If it can be assumed that the sulfur compounds selected are representative of their classes, it is evident from the table that cyclic

Table V. Separation Data for Mixtures of Sulfur Compounds and Sulfur Compounds and Hydrocarbons

Components	Vol. %	B. P., °C.	Density, 20° C.	Mol. Wt.	Mol. Vol.	n_D^{20} Detd.	n_D^{20} API	Time in Column, Hours	Composition, Vol. %				Degree of Separation[a]
									Fr. 1	Fr. 2	Fr. 9	Fr. 10	
Iso-octane	95	99.238	0.69192	114	164.8	1.39151	1.39145	...	...	...	...	...	
1-Butanethiol	5	98.46	0.84161	90	106.9	1.4431	1.44298	108	...	...	...	...	No separation
n-Octane	90	125.665	0.70252	114	162.3	1.39774	1.39743	...	97.1	...	...	...	
1-Butanethiol	10	98.46	0.84161	90	106.9	1.4431	1.44298	141	...	...	...	38.7	Fair
Iso-octane	90	99.238	0.69192	114	164.8	1.39151	1.39145	...	98.9	98.9	...	...	
2,3-Dithiabutane	10	109.6	1.06250	94	88.5	1.52565	1.52592	108	...	...	20	51	Good
n-Octane	90	125.665	0.70252	114	162.3	1.39774	1.39743	...	99	...	...	...	
3,4-Dithiahexane	10	152.6	0.99311	122	122.8	1.50696	1.50731	257	...	...	...	78	Good
4-Thiaheptane	50	142.83	0.8377	118	140.9	1.44876	1.4487	...	95.7	...	...	4.1	
3,4-Dithiahexane	50	152.6	0.99311	122	122.8	1.50607	1.50731	145	4.3	...	...	95.9	Good
3,4-Dithiahexane	50	152.6	0.99311	122	122.8	1.50696	1.50731	...	63.4	...	...	42.1	
Thiacyclohexane	50	141.75	0.9856	102	103.5	1.50555	1.5067	141	36.6	...	...	58.0	Very poor
Ethylbenzene	50	138.186	0.86702	106	122.3	1.49578	1.49588	...	99.3	99.3	...	...	
Thiacyclohexane	50	141.75	0.9856	102	103.5	1.50641	1.5067	144	...	...	98.1	99.4	Excellent
Ethylbenzene	50	138.186	0.86702	106	122.3	1.49587	1.49588	...	43.5	44.9	59.5	63.2	
4-Thiaheptane	50	142.83	0.8377	118	140.9	1.44911	1.4487	166	56.5	55.1	40.5	36.8	Very poor
Iso-octane	95	99.238	0.69192	114	164.8	1.39151	1.39145	...	...	99.2	98.1	...	
Thiacyclohexane	5	141.75	0.9856	102	103.5	1.50641	1.5067	115	...	...	...	40.8	Good
Benzene	50	80.100	0.87901	78	88.7	1.50030	1.50112	...	76	...	...	25	
Thiophene	50	84.16	1.06485	84	78.9	1.52856	1.52890	149	24	...	...	75	Fair
Thiacyclohexane	50	141.75	0.9856	102	103.5	1.50590	1.5067	...	...	...	...	...	
2-Methylthiophene	50	112.56	1.0193	98	96.1	1.51983	1.52035	142	...	...	...	...	No separation
4-Thiaheptane[b]	50	142.83	0.8377	118	140.9	1.44900	1.4487	...	71.2	...	Fract. 4	28.6	
2-Methylthiophene	50	112.5	1.01965	98	96.1	1.51975	1.52035	168	28.8	...		71.4	Fair
1-Pentanethiol	50	126.64	0.84209	104	123.5	1.44762	1.44692	...	95.1	94.2	...	...	
3,4-Dithiahexane	50	152.6	0.99311	122	122.8	1.50694	1.50731	168	...	...	94.0	94.9	Excellent
1-Pentanethiol	50	126.64	0.84209	104	123.5	1.44762	1.44692	...	99.5	99.5	1.1	0.7	
Thiacyclahexane	50	141.75	0.9856	102	103.5	1.50641	1.5067	144	0.5	0.5	98.9	99.3	Excellent
2,3-Dithiabutane	50	109.6	1.06250	94	88.5	1.52565	1.52592	...	...	...	...	...	
Thiophene	50	84.16	1.06485	84	78.9	1.52890	1.52890	120	...	...	...	...	Poor

[a] Based not only on data reported in columns 10–13 but also on nature of refractive index–volume per cent curves and other factors.

[b] Experiment conducted in 18-inch column—four fractions collected, each representing 25% of sample.

Table VI. General Conclusions on Separability of Sulfur Compounds by Thermal Diffusion

Family	Typified by	Comment on Separation
Cyclic sulfides–chain sulfides	Thiacyclohexane–4-thiaheptane (normal)	Excellent
	Thiacyclohexane–2,8-dimethyl-5-thianonane (isoamyl)	Excellent
	Thiacyclohexane–3,5-dimethyl-4-thiaheptane (*sec*-butyl)	Excellent
Cyclic sulfides–aromatics	Thiacyclohexane–ethylbenzene	Excellent
Cyclic sulfides–disulfides	Thiacyclohexane–3,4-dithiahexane	Very poor
Cyclic sulfides–thiophenes, substituted	Thiacyclohexane–2-methylthiophene	None
Chain sulfides–cyclic sulfides		Excellent
Chain sulfides–chain sulfides	4-Thiaheptane–2-thiaheptane	Fair
Chain sulfides–aromatics	4-Thiaheptane–ethylbenzene	Very poor
Chain sulfides–disulfides	4-Thiaheptane–3,4-dithiahexane	Good
Chain sulfides–thiophenes, substituted	4-Thiaheptane–2-methylthiophene	Fair
Amyl sulfides–amylcyclopentyl sulfides	*n*-Amyl sulfide–amylcyclopentyl sulfide	Good
Elemental sulfur–aromatics	Elemental sulfur–ethylbenzene	Excellent
	Elemental sulfur–cumene	Excellent
Disulfides–thiophenes	2,3-Dithiabutane–thiophene	Poor
Aliphatic thiols–disulfides	1-Pentanethiol–3,4-dithiahexane	Excellent
Aliphatic thiols–cyclic sulfides	1-Pentanethiol–thiacyclohexane	Excellent

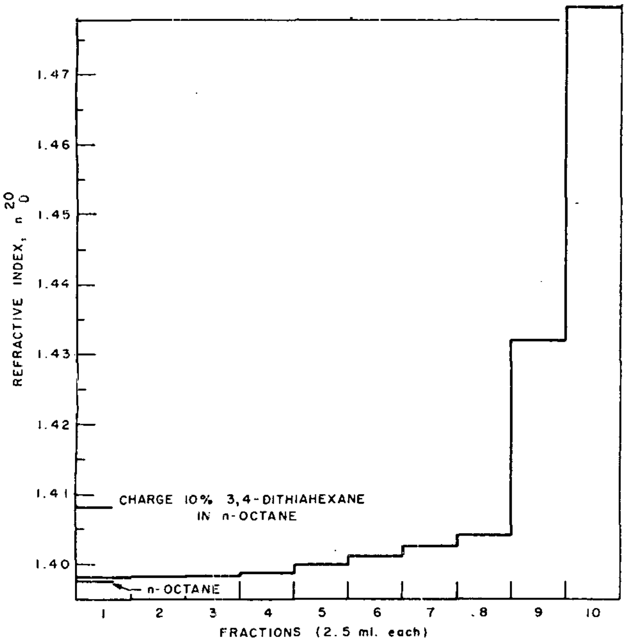

Figure 9. Concentration of 3,4-dithiahexane in *n*-octane by liquid thermal diffusion

6-foot stainless steel column
Hot wall temp., 54° C.
Cold wall temp., 28° C.
Time in column, 257 hours

Fraction No.	Constituent	%
1	*n*-Octane	99
10	3,4-Dithiahexane	78

sulfides and substituted thiophenes cannot be separated. Poor separations are realized with mixtures of cyclic sulfides and disulfides. Fair to good separations are achieved with mixtures of chain sulfides and substituted thiophenes and chain sulfides and disulfides. Poor separations are realized with mixtures of thiophenes and disulfides. Excellent separations are obtainable between thiols and cyclic sulfides, and thiols and disulfides. These general conclusions from the data in Tables IV and V are summarized in Table VI.

Most sulfur compounds when blended to about 10 volume % in *n*-octane or iso-octane can be concentrated or separated from these solvents with fair to good success, as shown by the example in Figure 9. Notable exceptions are 1-butanethiol and 4-thiaheptane, which will not concentrate in iso-octane but will concentrate to some extent in *n*-octane. Elemental sulfur can be concentrated to a high degree in aromatics such as ethylbenzene or cumene.

The general conclusions, summarized in Table VI, are based upon limited experimental data. As additional mixtures are investigated, exceptions to these generalities may become evident. Thermal diffusion will not replace adsorption and distillation techniques, but as the method becomes refined and the potentialities of the process become more widely known it should prove to be a powerful supplement to these other separation processes.

Thermal Diffusion *vs.* Adsorption. In an investigation, such as the work of project 48A, which requires the separation of sulfur compounds from petroleum, it is evident that no single process or technique will accomplish the objective completely. Many tools and methods must be applied, each in the proper sequence, depending upon the requirements of the problem.

A comparison of some of the available methods should be useful in the establishment of an over-all procedure. In 1949 Smit (*28*) reported application of thermal diffusion and adsorption to a completely hydrogenated lubricating oil. For this particular type of separation, adsorptive percolation gave somewhat superior results to those obtained by thermal diffusion.

To serve as a guide in separating sulfur compounds, a comparison was made of the relative efficiency of separations obtainable by adsorption and thermal diffusion. Experiments were conducted by both processes on identical synthetic mixtures.

The adsorption experiments were made in small-bore, long columns containing 250 ml. of fluid grade H-41 alumina. These columns consisted of three sections of the following dimensions (top to bottom): 75 cm. long, 15 mm. in diameter; 80 cm. long, 11 mm. in diameter; and 120 cm. long, 7 mm. in diameter. For this particular set of comparisons the complete displacement technique was utilized. Ten milliliters of sample (5 ml. of each component) diluted with 40 ml. of iso-octane was charged to the alumina and followed by the desorbent ethyl alcohol. Because the average particle size of the H-41 fluid grade alumina used in these tests was only 50 microns, about 48 hours was required to complete each adsorption experiment. Small fractions were taken and by carefully following boundaries very sharp "cut points" were realized.

Table VII gives the mixtures tested and the degree of separation obtained by each process. Further comparison of the two processes is presented in Figures 10, 11, and 12. As shown in Figure 10, adsorption is definitely better than thermal diffusion in the separation of 50–50 volume % mixtures of 4-thiaheptane and ethylbenzene. Conversely, in Figure 11 thermal diffusion is superior to adsorption in the separation of (1-thiahexyl) cyclopentane and 6-thiaundecane. In the third example (Figure 12) there is little choice between the two procedures.

Each process has advantages and disadvantages peculiar to itself. Adsorption, for example, usually requires less over-all time but more attention during the time involved, whereas thermal diffusion requires more over-all time but less attention during

Table VII. Adsorption *vs.* Thermal Diffusion

Mixture	Degree of Separation Achieved	
	Adsorption	Thermal diffusion
Thiacyclohexane–4-thiaheptane	Excellent	Excellent
Thiacyclohexane–ethylbenzene	Excellent	Excellent
4-Thiaheptane–ethylbenzene	Excellent	Very poor
(1-Thiahexyl)cyclopentane–6-thiaundecane[a]	Poor	Good
4-Thiaheptane–2-thiaheptane	Very good	Fair
4-Thiaheptane–3,4-dithiahexane	Fair to good	Fair to good
2-Methylthiacyclopentane–2-thiaheptane Thiacyclohexane–4-thiaheptane	Excellent	Excellent

[a] Accuracy of analytical data limited by presence of impurities.

the processing. In the example shown in Figure 12, adsorption gives a greater yield of pure product than does thermal diffusion, although it should be acknowledged that these adsorption experiments were made more carefully than usual and the degree of separation indicated is not always achieved in every column. From these considerations, it is apparent that neither method can be said unreservedly to have advantage over the other for separating all mixtures. The method of separation for any particular mixture must be considered individually.

The present interest in sulfur compounds in crude oil continues to shift to the distillates of higher boiling range, and the problems incurred in isolating or identifying sulfur compounds as they occur in the original crude oil continue to increase in difficulty. This paper has shown that distillation, adsorption, and thermal diffusion techniques are very effective for separating sulfur compounds, and combinations of these techniques appear to be applicable, with various modifications, not only to all boiling range distillates but to the complete crude oil.

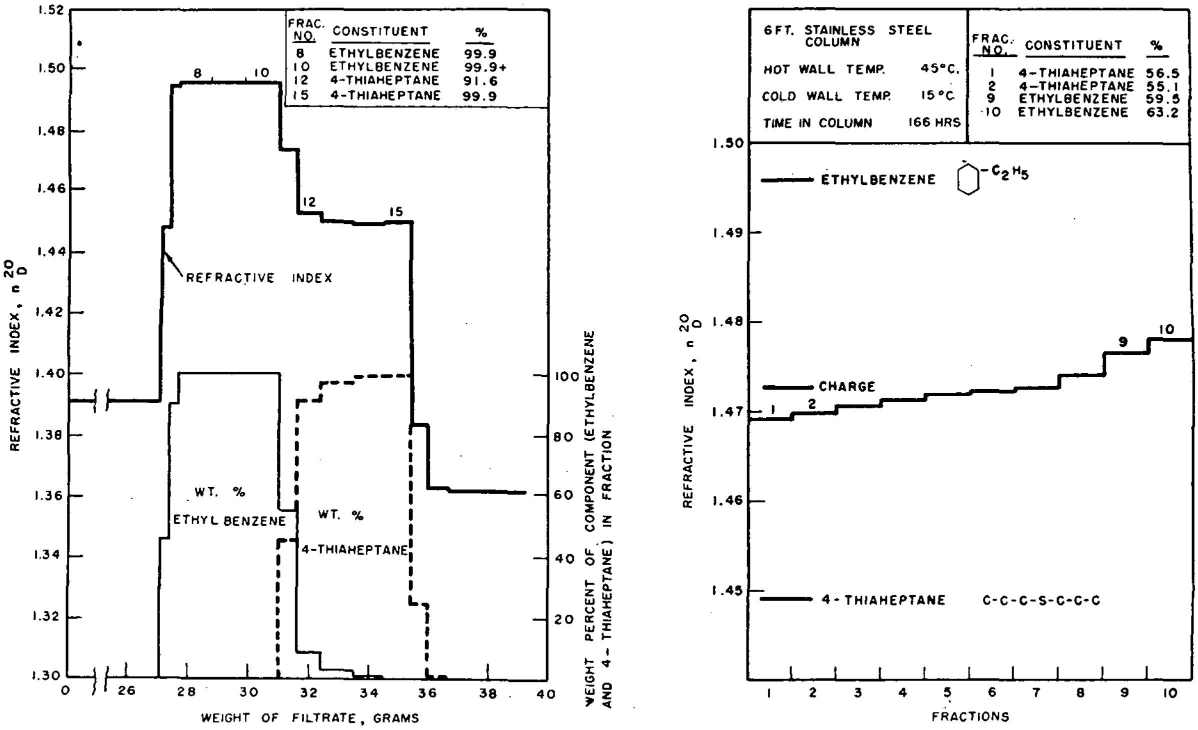

Figure 10. Comparison of adsorption and liquid thermal diffusion in separating 50–50 volume % blend of ethylbenzene and 4-thiaheptane

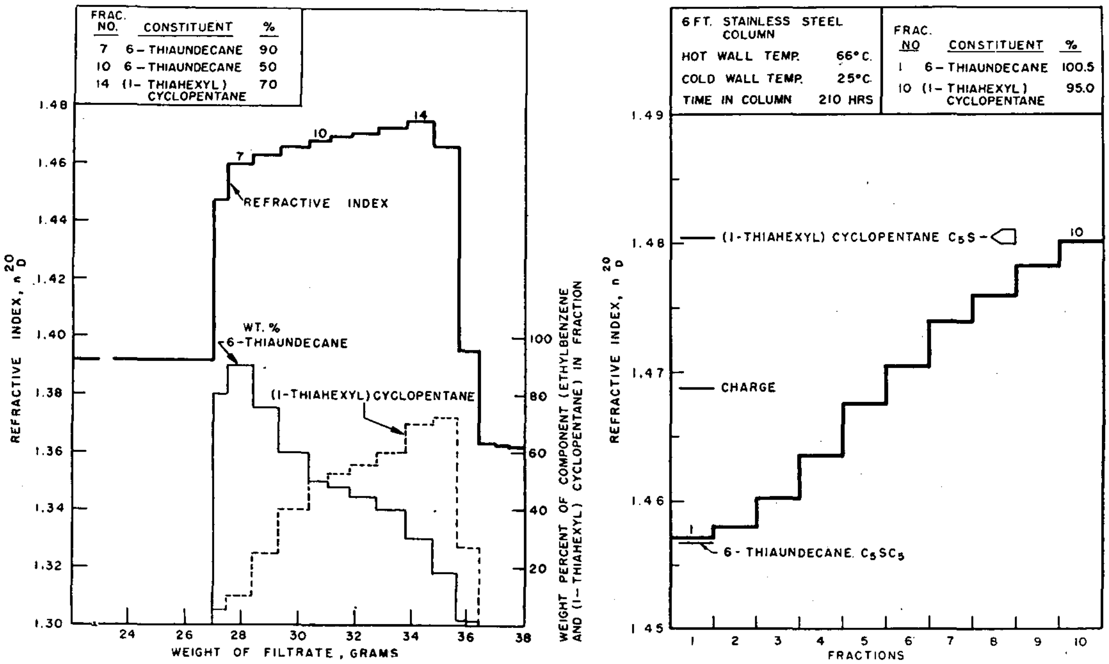

Figure 11. Comparison of adsorption and liquid thermal diffusion in separating 50–50% blend of 6-thiaundecane and (1-thiahexyl)cyclopentane

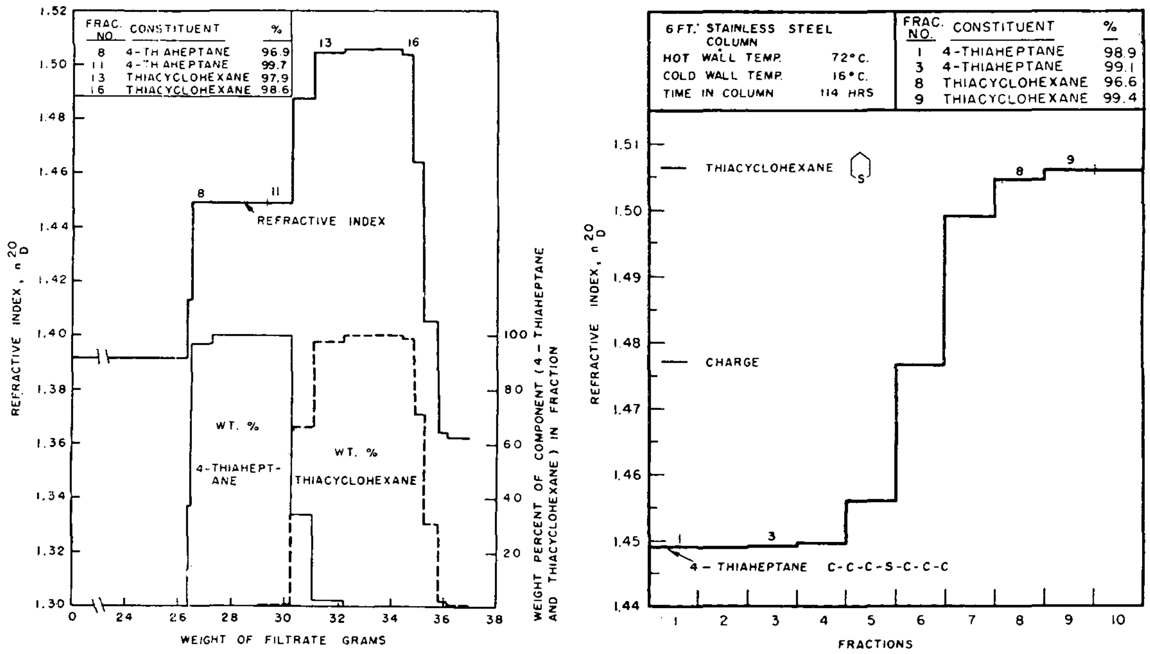

Figure 12. Comparison of adsorption and liquid thermal diffusion in separating 50–50% blend of 4-thiaheptane and thiacyclohexane

SULFUR COMPOUNDS IDENTIFIED IN WASSON CRUDE OIL

By the application of various distillation techniques, adsorption, and chemical treatment as described in this paper and others (*3*, *13*) 43 sulfur compounds have been identified in Wasson, Tex., crude oil. Table VIII records these compounds, their boiling points, and estimated percentages present.

SUMMARY

The work of the American Petroleum Institute and others has shown that physical methods of separation offer the most promise of separating sulfur compounds unchanged from a crude oil or its fractions. Chemical methods can be used more advantageously for sulfur compounds than for hydrocarbons because of the greater reactivity of the sulfur compounds, but this factor also limits the usefulness of chemical methods, because some reactions destroy the original compound. Chemical methods probably will find their greatest usefulness in separating different types of sulfur compounds from each other after they have been separated from hydrocarbons.

Of the physical methods of separation, distillation can be used only under limited conditions such that thermal decomposition is avoided—as in the isothermal preparation of low boiling distillates and the fractionation of concentrates of similar sulfur compounds. The most effective separative means employed to date has been chromatography on alumina. This has been effective when applied to distillates and to a topped deasphaltened crude oil. Thermal diffusion has been investigated as a separative tool and for some separations offers distinct advantages. It seems probable that the carefully integrated use of adsorption and thermal diffusion will permit many effective separations of types of sulfur compounds.

Using only distillation, within the limits required to avoid decomposition, and adsorption on alumina, it has been possible to prepare fractions and identify by infrared and mass spectra 43 sulfur compounds boiling below 220° C. In addition, by chromatography of a topped deasphaltened crude oil it has been established that thiophenes with two, three, and four or more rings constituted at least 65% of the 15% of sulfur compounds in this major part of the crude oil.

Table VIII. Sulfur Compounds Identified in Wasson, Tex., Crude Oil and Estimated Weight Per Cent Present Where Known

Name	Boiling point, °C.	Weight % in Crude Oil[a]	Weight % of Total Sulfur in Crude Oil
Methanethiol	5.96	0.00240	0.0865
Ethanethiol	35.0	0.00530	0.1478
2-Thiapropane	37.31	0.00088	0.0245
2-Propanethiol	52.56	0.00199	0.0453
2-Methyl-2-propanethio	64.22	0.00055	0.0106
2-Thiabutane	66.65	0.00222	0.0505
1-Propanethiol	67.5	0.00041	0.0093
3-Methyl-2-thiabutane	84.81	0.00064	0.0123
2-Butanethiol	85.15	0.00386	0.0742
2-Methyl-1-propanethiol	88.72	0.00003	0.0006
3-Thiapentane	92.10	0.00075	0.0144
2-Thiapentane	95.52	0.00030	0.0058
1-Butanethiol	98.4	Trace	Trace
2-Methyl-2-butanethiol	99.0	0.00064	0.0106
3,3-Dimethyl-2-thiabutane	99.0	...	...
2-Methyl-3-thiapentane	107.4	...	...
3-Methyl-2-butanethiol	109.8	...	...
2-Pentanethiol	112.9	0.0014	0.023
3-Pentanethiol	113.9	0.00057	0.0095
4-Methyl-2-pentanethiol	...	...	...
3-Methyl-3-pentanethiol	...	...	...
3-Thiahexane	118.50	0.00012	0.0020
2,4-Dimethyl-3-thiapentane	120.02	0.00053	0.0078
2,2-Dimethyl-3-thiapentane	120.41	0.000058	0.0008
Thiacyclopentane	121.12	0.000077	0.0015
2-Thiahexane	123.2	0.000077	0.0013
2-Methyl-3-thiahexane	132.05	0.000078	0.0011
Cyclopentanethiol	132.2	...	...
2-Methylthiacyclopentane	133.23	0.0023	0.039
4-Methyl-3-thiahexane	133.65	0.00050	0.0073
3-Methylthiacyclopentane	138.67	0.00046	0.0078
2-Hexanethiol	138.9	0.0028	0.041
Thiacyclohexane	141.75	0.00032	0.0054
trans-2,5-Dimethylthiacyclopentane	142.0	0.0025	0.037
cis-2,5-Dimethylthiacyclopentane	142.28	0.0024	0.036
3-Thiaheptane	144.24	0.000078	0.0011
2-Methylthiacyclohexane	153.04	0.0029	0.043
3-Methylthiacyclohexane	158.04	0.000024	0.0004
4-Methylthiacyclohexane	158.64	0.000048	0.0007
Cyclohexanethiol	158.8	0.0012	0.018
Tentatively Identified			
3-Hexanethiol			
cis-2-Methylcyclopentanethiol			
2-Octanethiol			
			0.78

[a] Minimum values. For thiols and sulfides boiling below 100° C. and for thiols boiling above 100° C. values should be 90–95% correct. Values reported for cyclic and chain sulfides boiling above 111° C. may be low by 50% because of processing losses.

ACKNOWLEDGMENT

The authors wish to acknowledge the contribution of Louis Mikkelsen and Dorothy Richardson in supplying the infrared and part of the mass spectral data reported in this paper.

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RECEIVED for review July 28, 1954. Accepted December 27, 1954.

Application of Separation Techniques to a High-Boiling Shale-Oil Distillate

G. U. DINNEEN, J. R. SMITH[1], R. A. VAN METER, C. S. ALLBRIGHT, and W. R. ANTHONEY[2]

Petroleum and Oil-Shale Experiment Station, Bureau of Mines, Laramie, Wyo.

Modern separation techniques have been used to resolve a gas oil from Colorado shale oil into simpler fractions to facilitate studies of composition. The gas oil was a complex material containing about equal quantities of hydrocarbon and nonhydrocarbon compounds. Adsorption techniques were the principal tools used, supplemented by vacuum distillation, thermal diffusion, and adduct formation. These techniques were selected to provide maximum resolution and recovery of material and to minimize decomposition. The gas oil was separated into nitrogen, aromatic, olefinic, and saturated concentrates. These were further separated into simpler fractions. Information presented in this paper should be applicable to composition studies in other fields.

MODERN separation techniques have been used to resolve a gas oil from Colorado shale oil into simpler fractions to facilitate composition studies. Adsorption utilizing a variety of adsorbents was the principal tool used, supplemented by vacuum distillation, thermal diffusion, and adduct formation. These techniques were selected to give maximum resolution and recovery of material, and to minimize decomposition, so the results could be more easily related to the original gas oil.

Crude shale oil obtained by most retorting processes from Colorado shale contains only a small quantity of material boiling in the gasoline range. Accordingly, production of volatile fuels utilizes the higher boiling fractions as raw material, and therefore knowledge of their composition is important. The separation techniques discussed in this paper were investigated for use on a gas-oil fraction having a boiling range of 625° to 1100° F. It had been distilled from a crude shale oil produced from Colorado shale in an N-T-U retort at Rifle, Colo. The gas oil contained about 1.8% nitrogen, 0.6% sulfur, and 1.0% oxygen. As the determination of individual compounds in such a mixture is difficult, major emphasis was placed on the identification of types of compounds. This required separation of the material into more or less homogeneous fractions.

A sample of 16 liters of the gas oil was first separated, by an adsorption technique utilizing Florisil, into two concentrates, one containing most of the nitrogen compounds originally present in the distillate, and the other containing predominantly hydrocarbons. The hydrocarbon concentrate was then further separated into concentrates of aromatic, olefinic, and saturated compounds by a two-step process, employing silica gel as the adsorbent. The aromatics were first removed from the saturates and olefins. These latter compounds were then separated from each other, using a much higher adsorbent-sample ratio than was used to remove the aromatics. Further separations on these primary concentrates were made by vacuum distillation, thermal diffusion, adduct formation, or additional adsorption techniques.

SEPARATION OF GAS OIL

Resolution of the gas oil into reasonably simple fractions was effected by a number of consecutive separation steps. An out-

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[1] Present address, Stanford Research Institute, Stanford, Calif.
[2] Present address, Tide Water Associated Oil Co., Associated, Calif.

line of the techniques employed and of the quantities of the major fractions obtained is given in Figure 1. As indicated in the figure, adsorption was the principal tool used, other techniques being applied after major separations had been accomplished.

Adsorption. The gas oil was a complex material containing substantial quantities of both hydrocarbons and nonhydrocarbons, including several distinct classes of each of these groups. For such material it seemed desirable to make the primary separations by types of compounds rather than on molecular size or shape. Adsorption and extraction are the most promising techniques for separating classes of compounds. Adsorption was selected because it usually gives sharper separations and more nearly quantitative recoveries than extraction, although the procedure may not be so simple and rapid.

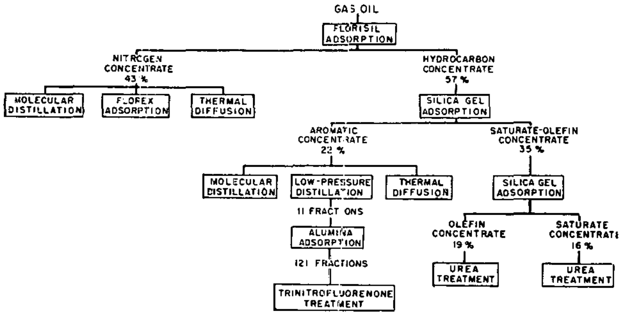

Figure 1. Outline of separation techniques used on gas oil

Florisil. Successful concentration of the nitrogen required the use of an adsorbent having a selective affinity for compounds containing this element. Experiments in small scale glass columns showed (*8*) that Florisil, a synthetic magnesium silicate manufactured by the Floridin Co., was a superior adsorbent for this purpose. Under the conditions of these experiments the Florisil retained 0.65 gram of nitrogen per 100 grams of adsorbent. This was about 50% more than was retained by the best other adsorbents tested. An additional advantage of Florisil was the rapid and more nearly quantitative recovery of the nitrogen compounds by desorption with acetone or methanol.

Separation of 16 liters of gas oil on Florisil was accomplished in the equipment shown schematically in Figure 2. A summary of the operating conditions used is given in Table I. Both acetone and methanol were tried as desorbents for the nitrogen concentrate. The recoveries were equivalent, but acetone was selected because of its superior operational characteristics: more rapid passage through the column and consequent reduction in the time required for a run; less desorption of water from the Florisil, so stripping of the desorbent from the eluate was simplified; and easier removal of the last traces of desorbent from the concentrate.

The data in Table II show the separation accomplished. The total recovery of nitrogen was 260.4 grams or about 90%, and all but 5.3 grams was in the nitrogen concentrate. The separation had little effect on the sulfur content, as the nitrogen concentrate contained only slightly more sulfur than did the hydrocarbon concentrate.

Silica Gel. The hydrocarbon concentrate obtained by elution with pentane from Florisil was separated into an aromatic concentrate and a saturate-olefin concentrate, using silica gel (*3*). The separations were made in two sets of equipment similar to that shown in Figure 2, except that each column consisted of 54 feet of 3/4-inch stainless steel tubing.

A typical run for preparing the aromatic concentrate employed 4200 grams of 28- to 200-mesh silica gel and a 500-gram charge of hydrocarbon concentrate. To accomplish the separation successfully with this adsorbent-sample ratio required that the sample be dissolved in about 3 volumes of pentane before being introduced into the column. Presumably, at least this amount was necessary to prevent solidification of some of the components as they were separated. However, higher dilutions were avoided, as they undesirably delayed entrance of all the sample into the adsorbent. The column of adsorbent was washed with pentane to remove the saturate-olefin concentrate and then with acetone to remove the aromatic concentrate. The results obtained by the separation are shown in Table III. Most of the sulfur and all of the nitrogen in the hydrocarbon concentrate were obtained in the

Table I. Conditions for Preparation of Nitrogen Concentrate

Total number of runs	23
Adsorbent	5 kg. of Florisil, 30–60 mesh
Sample	700 grams of gas oil dissolved in 2500 ml. of pentane
Pressure	20 lb./sq. inch gage
Eluant for hydrocarbons	15 liters of pentane
Desorbent for nitrogen concentrate	5 liters of acetone

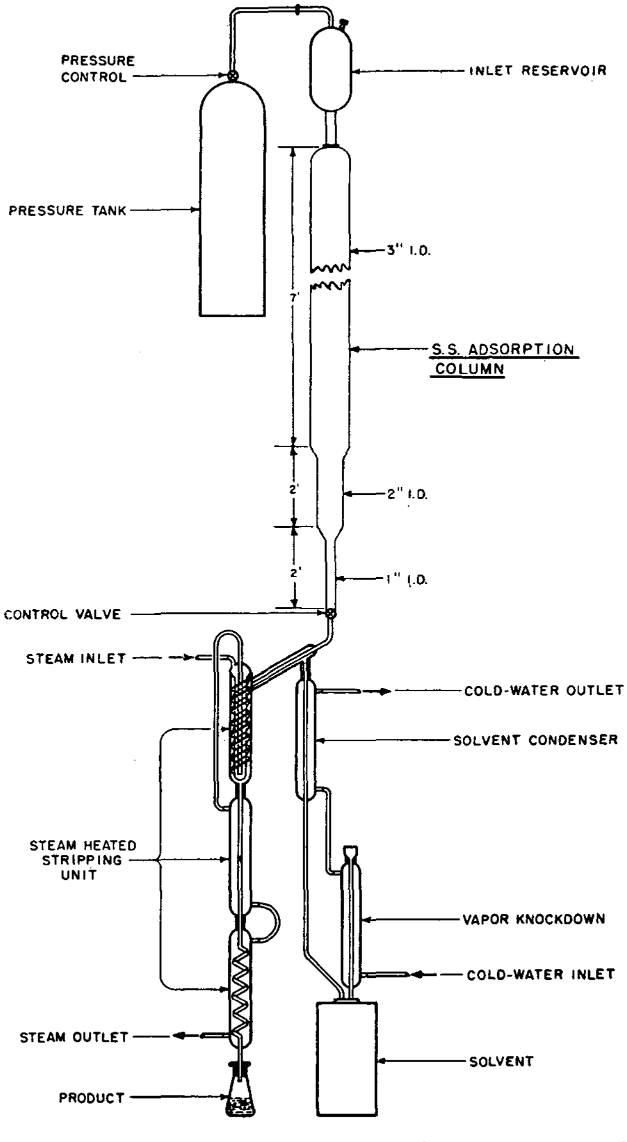

Figure 2. Adsorption equipment for separation of gas-oil fraction

Table II. Preparation of Hydrocarbon and Nitrogen Concentrates

	Material Balance		Nitrogen		Sulfur	
	Weight %	Grams	Weight %	Grams	Weight %	Grams
Charge	100.0	16,121	1.79	288.6	0.57	91.9
Nitrogen concentrate	41.8	6,732	3.79	255.1	0.68	45.8
Hydrocarbon concentrate	55.1	8,887	0.06	5.3	0.51	45.3
Loss	3.1	502		28.2		0.8

Table III. Preparation of Aromatic Concentrate

	Material Balance		Sulfur		Nitrogen	
	Weight %	Grams	Weight %	Grams	Weight %	Grams
Charge	100.0	8501	0.51	43.4	0.06	5.1
Aromatic concentrate	32.5	2766	1.47	40.7	0.18	5.0
Saturate-olefin concentrate	62.2	5288	0.03	1.6	0.00	...
Loss	5.3	447		1.1		0.1

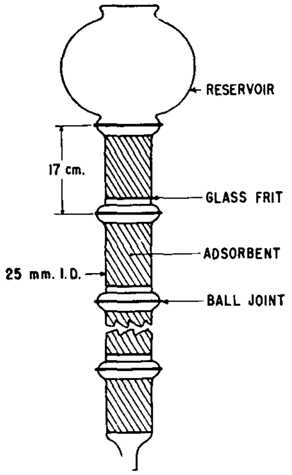

Figure 3. Multiple-section adsorption column

aromatic concentrate. Analytical work indicated that the saturate-olefin concentrate contained about 2% aromatics.

Silica gel was used to separate the saturate-olefin concentrate into saturates and olefins. This separation could have been accomplished in the previous operation, but the use of successive steps reduced the time required by about 50%. The one-step process would require treatment of the total hydrocarbon concentrate at an adsorbent-sample ratio somewhat greater than the 60 necessary for the saturate-olefin separation. However, by the two-step process the aromatics, amounting to about one third of the hydrocarbon concentrate, were removed at the low adsorbent-sample ratio of 8, so that only about two thirds of the concentrate needed to be processed at a ratio of 60. Furthermore, the technique used for the saturate-olefin separation permitted several runs to be made through the same batch of adsorbent without cleaning and recharging the column.

The separation of the saturates from the olefins required approximately 4200 grams of silica gel to 70 grams of sample per run. The diluted sample was placed on gel prewet with pentane, and eluted with approximately 8 liters of pentane to remove the saturates. The product receiver was changed, and the olefins were eluted with about 10 liters of pentane. Thus, by elution with pentane all of the sample was removed from the column in two distinct fractions. The first was primarily saturated, whereas the second was largely olefinic. Several successive charges of sample were separated on the same batch of adsorbent. Because of the presence of a small amount of aromatic material in the charge, the process could not be continued indefinitely. Six runs were usually made on a single filling of adsorbent. The aromatic material that accumulated on the adsorbent during the series of runs was recovered and added to the aromatic concentrate.

The recoveries and properties of the concentrates prepared in this separation are shown in Table IV. The properties of the saturate and olefin concentrates are approximately those that would be expected of compounds of the types indicated by the names, an indication that the separation was reasonably effective in preparing the type of concentrate desired.

ALUMINA. The aromatic concentrate contained hydrocarbons of a number of structural types as well as the sulfur and oxygen compounds in the hydrocarbon concentrate. It would be advantageous to separate compounds containing different numbers of condensed aromatic rings. Alumina is a good adsorbent for this purpose (*5*). However, the separation is improved if the charge stock has a relatively narrow range of molecular sizes; the aromatic concentrate was therefore distilled at low pressure into 10 distillate fractions and a residue (see section on distillation).

Each of the distillation fractions was developed on alumina in the 10-section column (*1*) shown in Figure 3. The operation is outlined in Table V. This technique gave arbitrary divisions, so fractions in adjacent sections of the column were often similar. Ultraviolet spectra on the various fractions indicated a fairly sharp separation between materials having different numbers of aromatic rings.

FLOREX. The nitrogen concentrate contained approximately 3.8 weight % nitrogen, 60% of which could be titrated as basic material. Extraction with aqueous mineral acid did not give a satisfactory separation of this type of nitrogen as a yield of only about 6.5% extract was obtained. Additional adsorption techniques were investigated as a means of separating basic from nonbasic nitrogen.

Table IV. Separation of Saturate-Olefin Concentrates

	Material Balance		Refractive Index, n_D^{84}	Molecular Weight
	Weight %	Grams		
Charge	100.0	4323	1.4378	
Olefin concentrate	43.2	1910	1.4458	357
Saturate concentrate	53.9	2385	1.4292	359
Aromatics returned to aromatic concentrate	2.1	91		
Loss	0.8	37		

Table V. Conditions for Alumina Separation of Aromatic Concentrate

Adsorbent	H-41 alumina, 14-mesh
Quantity per section	37 grams
Sample	15 ml. of fraction diluted to 100 ml. with pentane
Eluant	Pentane
Desorbent	Acetone

In the original preparation of the nitrogen concentrate, a Florisil-sample ratio of about 7 was used. An attempt to fractionate the nitrogen concentrate further on this adsorbent by increasing the ratio to 30 gave unsatisfactory results, as shown in Table VI. Rather prolonged elutions with pentane and benzene yielded only 15% of the nitrogen concentrate in the eluate, but 5% methanol in benzene desorbed three fourths of the sample. Consideration of these results suggested that a less active adsorbent might be more suitable. Florex (an extruded fuller's earth manufactured by the Floridin Co. For analytical use, a material prepared especially to avoid contamination with organic matter should be used.) was selected because previous experiments had shown that it would retain about 65% as much

Table VI. Fractionation of Nitrogen Concentrate by Adsorption on Florisil

Fraction No.	Eluant		Yield		Nitrogen, Weight %	
	Composition	Volume, ml.	Weight %	Grams	Total	Basic
Charge			100.0	6.0	3.79	2.10
1	Pentane	900	2.0	0.12		
2	Benzene	850	13.5	0.81		
3	5% methanol 95% benzene	1100	75.7	4.54	3.65	2.17

nitrogen as Florisil. A Florex-sample ratio of 20 for use in separating 115 grams of the nitrogen concentrate was selected, based on the results of small scale runs.

Fractionation of the nitrogen concentrate by gradual elution from Florex required a series of eluants of increasing eluting strengths. The pure compounds used in order of their eluting strengths were: pentane, benzene, 1,2-dichloroethane, acetone, and methanol. To obtain the desired gradual elution, binary mixtures were also used—for example, pentane was followed by pentane-benzene mixtures, then benzene alone, and so on. Experiments showed that in the Florex–gas oil system this technique gave satisfactory results with mixtures of pentane and benzene and of benzene and 1,2-dichloroethane. Less satisfactory results were obtained with mixtures containing acetone or methanol, as these compounds tended to desorb rather than elute the sample. Several olefins were investigated for use as eluants between pentane and benzene. Mixtures of the latter compounds were superior, largely because of the much greater ease of removing them from the eluate fractions.

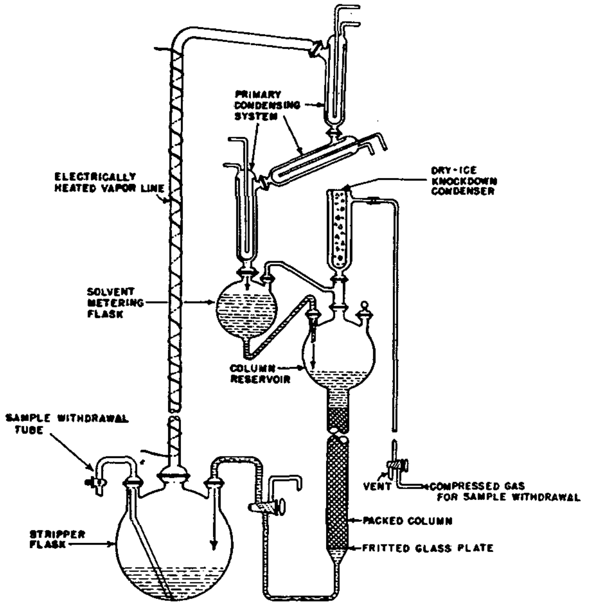

Figure 4. Adsorption column with solvent-recycle system

In the separation of 115 grams of nitrogen concentrate, 2300 grams of 30- to 60-mesh Florex was used. The glass adsorption column was 55 mm. in internal diameter and 1.7 meters long. Elution of the individual fractions required amounts of solvent ranging from 20 to 130 liters. Owing to the large volumes of solvents, equipment shown schematically in Figure 4 was constructed to provide continuous stripping and recirculation of the solvent, through a siphon metering device in order to measure the volume. A heated flask was selected as the stripping unit because it was thought operational advantages outweighed the disadvantage of maintaining the sample at the low boiling temperature of the solvents, particularly as fractions were to be removed at rather frequent intervals.

The sample of nitrogen concentrate dissolved in pentane was charged to the Florex-packed column and was developed with pentane. In general, fractions were cut at intervals estimated to yield 5% of the charge material per fraction. These estimates were based on measurements of ultraviolet absorbance at 3400 A. made on material in the stripper flask. Changes in elution-solvent composition to achieve graded increases in elution strength were dictated by a marked falling off of product yield with a particular solvent composition. These points usually could be judged by fading of color in the eluate stream. Twenty-three fractions were obtained. The basic and nonbasic nitrogen contents of each fraction are shown in Figure 5.

Although complete separation of these types of compounds was not achieved, the early fractions were much richer in nonbasic nitrogen, whereas the later fractions were rich in basic nitrogen. This distribution of types of nitrogen is shown in another way in Figure 6. The recovery of nonbasic nitrogen was approximately equal to the recovery of material for about the first 30%, but the recovery of basic nitrogen over this range was very low. Thereafter, the effect of the higher basic nitrogen content of the fractions was evident, and ultimate recovery of this type of nitrogen slightly exceeded that of the nonbasic. Elution solvent compositions are also shown in Figure 5. No clear-cut correlation between solvent and fraction compositions was evident.

Distillation. The separations described previously could be applied satisfactorily to fractions of wide boiling range because classes of compounds having distinctly different properties were involved. The concentrates obtained were still complex, so further separations were required before the ultimate aim of determining the composition of the material could be achieved. However, differences in properties on which separations within classes must be based may be small and are often partially nullified by

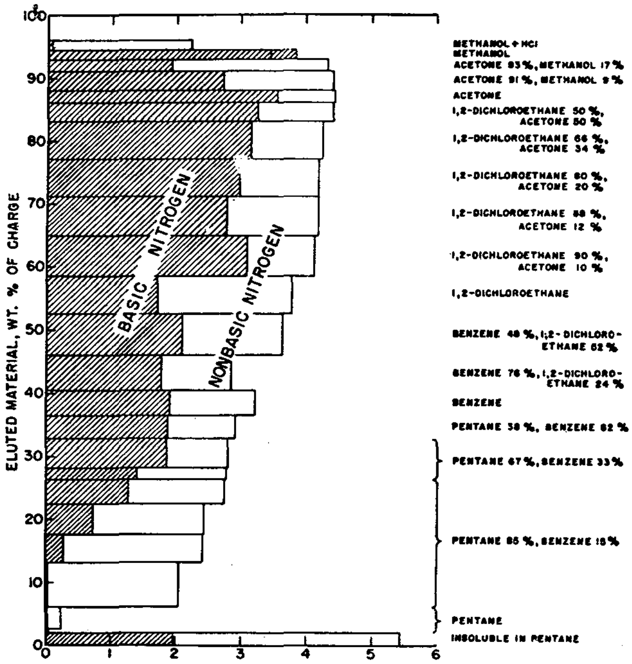

Figure 5. Distribution of nitrogen in fractions from nitrogen concentrate

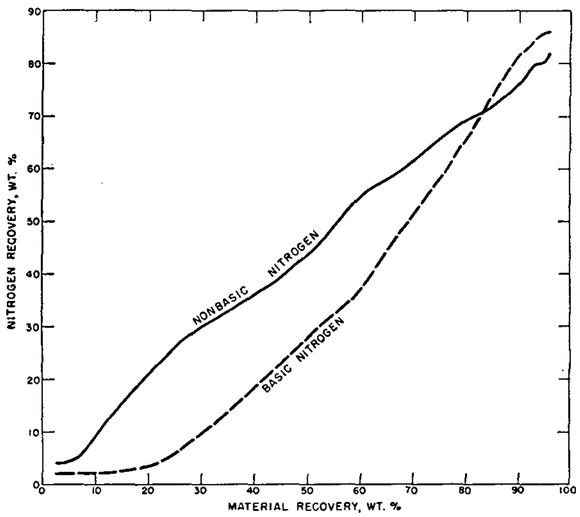

Figure 6. Comparison of material and nitrogen recoveries

molecular weight effects. For example, increased number of condensed rings in aromatics tends to increase adsorbability, but greater molecular size tends to decrease it. Therefore, distillation to make a molecular weight separation is necessary at some stage of the work on all of the concentrates. In the boiling range of the gas oil very low pressures are required for this operation to minimize decomposition. The two distillation techniques used are described for the aromatic concentrate.

Fractions for the alumina separation discussed under the section on adsorption were prepared by distilling about 300 ml. of the aromatic concentrate into 10 fractions and a residue. The distillation was conducted at an absolute pressure of 0.20 ± 0.05 mm. of mercury. Vapor temperatures at this pressure ranged from 130° to 205° C. The equipment consisted of a flask surmounted by a concentric-tube column with a Claissen-type head. Column dimensions were: length, 20 cm.; outside diameter, 30 mm.; and annular space, 3.5 mm. About 85% of the charge was obtained as distillate.

The other distillation utilized a centrifugal molecular still having a 5-inch rotor. Distillation of a 500-ml. sample of the aromatic concentrate was made at 10-micron pressure. Rotor temperatures from 70° to 180° C. in 10° increments yielded 12 overhead cuts. On the basis of physical properties, these were recombined into five cuts, which were refractionated in the same molecular still using smaller rotor temperature increments to give a total of about 83% overhead in 30 fractions. The extent of fractionation obtained by this technique is indicated by the data shown in Figure 7.

Thermal Diffusion. Compounds that are similar except for molecular configuration may often be separated by thermal diffusion. The technique has been applied so far only to the aromatic and nitrogen concentrates. The aromatic concentrate was charged to a 6-foot concentric-tube steel column having an annular space of 0.012 inch, 10 withdrawal ports, and a total capacity of 35 to 40 ml. (*2*). Average operating temperatures were 47° C. on the hot wall and 25° C. on the cold wall. The run was continued for 100 hours. The refractive index of the charge was 1.529 and of the top and bottom fractions was 1.510 and 1.540, respectively, indicating some separation of this wide boiling range material. A similar separation of the nitrogen concentrate gave top and bottom fractions whose mass spectra showed large differences. The mass spectrum of the top fraction was simpler than that of the bottom fraction and had as its predominant feature a series of groups of four peaks. In each group a peak differed from the corresponding peak in the preceding group by a mass of 14, suggesting the presence of only a few homologous series.

Complex Formation. The formation of chemical complexes may be utilized in two ways—resolution of types of compounds or isolation of individual compounds. The wide range of reagents and conditions available makes this a versatile technique, which is applicable to various types of hydrocarbons as well as to nonhydrocarbon materials. However, in most instances the results are better if the samples are not too complex.

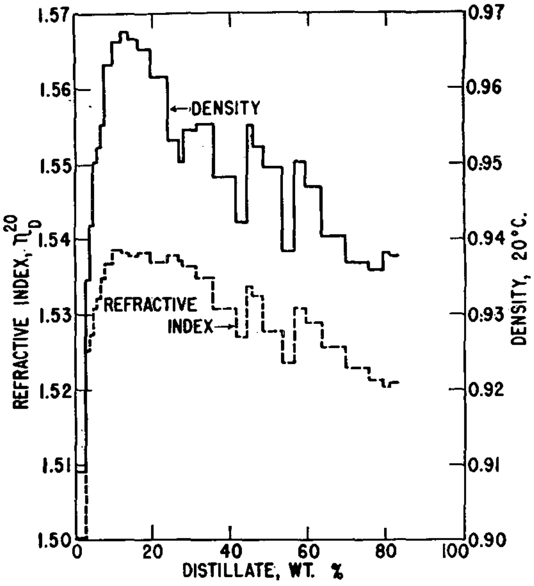

Figure 7. Properties of fractions from molecular distillation of aromatic concentrate

Urea forms a solid complex with compounds that contain a long unbranched hydrocarbon chain. In general, the complex is more stable the greater the number of carbon atoms in the chain and the fewer atoms in branched or cyclic structures to which the chain is attached. The reaction has been used frequently to estimate the extent of branching in molecules, although the results must be interpreted with caution. Each of the saturate and olefin concentrates was treated with urea (*7*). The separations were not made on a scale large enough to provide fractions for extensive evaluation but only as an estimate of chain branching. The predominantly straight-chain character of the molecules in each concentrate was indicated by the fact that approximately 85% of each formed an adduct with urea. Confirmation of the straight-chain character of the adducted compounds was obtained from infrared spectra, which showed the presence of about two methyl groups per molecule.

Several reagents, such as 2,4,7-trinitrofluorenone and picric acid, react with some aromatic compounds to give solid products. Several fractions from the alumina separation and from the molecular distillation were treated with 2,4,7-trinitrofluorenone (*6*). The fractions selected were those that ultraviolet spectra indicated to be richest in condensed-ring aromatics. Complexes were obtained on only a few of the fractions. Spectra indicated that material regenerated from the complex was richer in condensed-ring components than the original fraction but still contained a number of individual compounds. This was the case even on two fractions from which complexes with sharp melting points were obtained.

RESULTS OF SEPARATIONS

The separations discussed in this paper were made preparatory to identifying the types of compounds in the gas oil. Although the concentrates are still complex, some information concerning the gas oil can be obtained from them. The percentage of each concentrate in the gas oil, calculated on a no-loss basis, is shown

Table VII. Concentrates Prepared from Gas-Oil Fraction

	Gas Oil, Wt. %
Nitrogen concentrate	43
Aromatic concentrate	21
Olefin concentrate	16
Saturate concentrate	20

in Table VII. As the aromatic concentrate contains some sulfur and oxygen compounds, it can be seen that nonhydrocarbon compounds make up nearly half of the gas oil. Determinations on the nitrogen concentrate yielded the following data: nitrogen, 3.79%; oxygen, 2.55%; and molecular weight, 290. Calculation from these data indicates that many of the molecules in this concentrate must contain more than one atom other than carbon and hydrogen. The three classes of hydrocarbons are present in about equal amounts. From examination of the aromatic concentrate it appears that this material contains about equal quantities of one-, two-, and three-ring aromatic compounds. The saturate and olefin concentrates are predominantly straight-chain materials.

SUMMARY

A series of separation techniques, outlined in Figure 1, has been applied to a gas-oil distillate from shale oil. The techniques used were adsorption employing several different absorbents, vacuum distillation, thermal diffusion, and complex formation. The gas oil was a complex mixture of saturated, olefinic, and aromatic hydrocarbons, as well as nitrogen-, oxygen-, and sulfur-containing compounds. Hydrocarbons made up approximately half of the gas oil. This material was separated into a number of fractions, each of which was less complex than the original gas oil. Concentrates obtained were those composed of nitrogen compounds and aromatic, saturated, and olefinic hydrocarbons. The concentrates containing the saturated and olefinic materials were further subdivided into straight- and branched-chain compounds, and the aromatic concentrate was subdivided into fractions containing different numbers of condensed aromatic rings. For the nitrogen concentrate, separation was made into fractions containing different relative percentages of basic and nonbasic nitrogen.

ACKNOWLEDGMENT

This project was part of the Synthetic Liquid Fuels Program of the Bureau of Mines and was performed at the Petroleum and Oil-Shale Experiment Station under the direction of H. P. Rue, H. M. Thorne, and J. S. Ball. The authors wish to thank Minerva Landers and Elizabeth A. Pratt for analytical determinations in connection with this project. The work was done under a cooperative agreement between the University of Wyoming and the U. S. Department of the Interior, Bureau of Mines.

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RECEIVED for review July 26, 1954. Accepted December 20, 1954.

A Fifty-Stage Apparatus for Distillation at Very Low Pressures

BEVERIDGE J. MAIR, ARTHUR J. PIGNOCCO, and FREDERICK D. ROSSINI

Carnegie Institute of Technology, Pittsburgh, Pa.

This report describes the design, assembly, operation, and testing of a 50-stage apparatus for distillation at very low pressures, in the range 0.01 to 0.1 mm. of mercury. Results are presented for two distillations, one performed on a commercial mixture of normal paraffins C_{18} to C_{22}, and the other on a concentrate of normal paraffins C_{17} to C_{25} obtained from petroleum. Both distillations were carried out at a vapor pressure near 0.03 mm. of mercury, a throughput of 80 ml. (liquid) per hour, and a reflux ratio of 55 to 1, with a total distilling time near 1900 hours. Continuous distillation for about 6 hours was required to obtain a steady state in this 50-stage apparatus.

IN ITS work on fractionating hydrocarbons of higher molecular weight occurring in the heavy gas-oil and lubricant fractions of petroleum, the American Petroleum Institute Research Project 6 reported in 1929 and 1935 some simple apparatus for the distillation of such material at low pressures (*7*, *12*). Similar apparatus was used in the distillation at low pressures of the "water-white oil" and the "extract oil" portions of the lubricant fraction of petroleum in an investigation that was concluded in 1938 (*6*, *8*). It was realized, however, that really effective separation by distillation at very low pressures would require an apparatus involving many stages of separation.

In the intervening years, multistage apparatus for distillation at very low pressures have been described by Fawcett and McCowen (*3*), Wollner, Matchett, and Levine (*13*), Brewer and Madorsky (*2*), Madorsky, Bradt, and Strauss (*5*), Madorsky (*4*), Aldershoff, Booy, Langedijk, Philippi, and Waterman (*1*), and Melpolder, Washall, and Alexander (*9*).

The API Research Project 6 began in 1951 active work on the development of a multistage apparatus for distillation at very low pressures. This apparatus has been referred to in a preliminary way (*11*). The present report gives a detailed description of the design, assembly, operation, and testing of a 50-stage apparatus for distillation at very low pressures.

DESCRIPTION OF APPARATUS

Figure 1 shows the main part of the apparatus for distillation at very low pressures, consisting of the pot, *A*, the two halves of the rectifying section, *B*, and the reflux regulator, *C*. The rectifying section consists of gently sloping glass tubes (at an angle of about 3.5° to the horizontal) with internal stainless steel fittings arranged in such a manner as to constitute a succession of plates. The two halves, each containing 25 plates, are connected in series to give a total rectifying section of 50 plates.

Details of the pot and the lower portion of the rectifying section are shown in Figure 2.

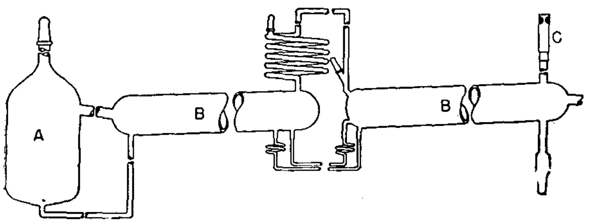

Figure 1. Schematic diagram of 50-stage apparatus for distillation at very low pressures

A.	Pot
B.	Rectifying section
C.	Reflux regulator

Details of the upper portion and of the reflux regulator are shown in Figure 3.

The cylindrical pot, *A* (Figure 2), made of borosilicate glass, contains an annular ring for collecting the distillate that condenses on the upper portion of the walls. The pot is connected to the rectifying section at two points. The upper connection, A_4, is for the passage of distillate from the collecting annulus to the rectifying section, *B*, and the lower connection, A_7, is for the passage of reflux from the rectifying section to the bottom of the pot. The outer containing tube of the rectifying section, B_1, is made of borosilicate glass with the lower side flattened. Inside this tube is a stainless steel sheet, B_2, curved to fit the walls and bent to form troughs, B_5. Successive plates are separated from each other by liquid-tight seals, B_4, in the troughs, B_5, and by vapor barriers, B_3, as well as by ridges, B_7, in the lower flattened side of the glass containing tube. For each plate the troughs have small stainless steel tubes, B_6, sealed as shown.

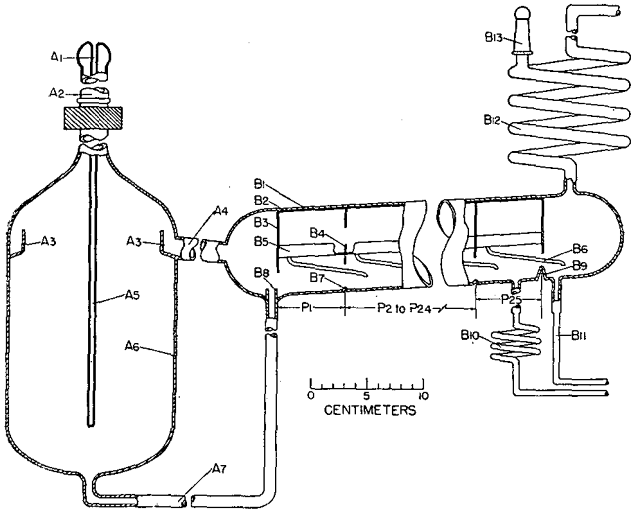

Figure 2. Lower portion of 50-stage apparatus for distillation at very low pressures

A.	Pot assembly
A_1.	Opening for thermocouple
A_2.	Opening for introduction of charge, with standard ground joint, 24/40
A_3.	Annular ring for collecting condensate
A_4.	Tube for carrying condensate to rectifying section
A_5.	Thermocouple well
A_6.	Glass pot
A_7.	Tube to return reflux to still pot
B.	Glass rectifying section, with internal stainless steel fittings
B_1.	Wall of rectifying section, 85-mm. O.D. glass tube
B_2.	Condensing surface, stainless steel, 0.02 inch thick
B_3.	Vapor barrier, stainless steel, 0.02 inch thick, spot-welded at one point to top of condensing surface
B_4.	Liquid barrier and spring strut, hard-soldered in gutter, stainless steel, 0.03 inch thick
B_5.	Gutter, to collect condensate
B_6.	Tube for carrying condensate to next higher plate
B_7.	Dam, 5/32 inch in height, for retaining some liquid at each plate
B_8.	Overflow, for returning liquid to still pot
B_9.	Dam, 1/2 inch in height, to provide liquid head required for condensate to pass to upper section
B_{10}.	Coiled tube, 6-mm. O.D., for return of reflux to bottom section
B_{11}.	Coiled tube, 6-mm. O.D., for passage of condensate to upper section
B_{12}.	Coiled tube, 10-mm. O.D., for evacuation of apparatus
P_1 to P_{25}.	Plates 1 to 25

Figure 4 gives additional details, with section *N* taken near the middle of a plate and section *M* at the end of a plate.

Connection between the two halves of the rectifying section is effected by the coiled glass tubes, B_{10}, B_{11}, and B_{12} (Figures 2 and 3). Tube B_{12} provides for the removal of air during the evacuation of the apparatus. Tube B_{11} provides for the passage of distillate from the top of the lower half of the rectifying section to the bottom of the upper half, while tube B_{10} provides for the return of reflux from the upper to the lower half of the rectifying section. The reflux regulator, consisting of the tapered glass stopper, C_3, the tungsten rod, C_2, and the control mechanism, C_1 (Figure 3), has been described (*10, 11*).

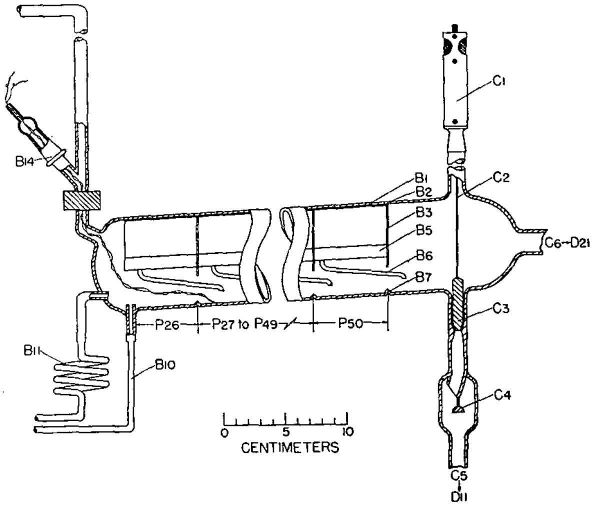

Figure 3. Upper portion of 50-stage apparatus for distillation at very low pressures

B_1 to B_7, B_{10}, and B_{11}	have significance indicated in Figure 2
B_{13}.	Opening for charging and cleaning rectifying section
B_{14}.	Thermocouple inlet with thermocouple to liquid in plate 27
P_{26} to P_{50}.	Plates 26 to 50
C.	Reflux regulator
C_1.	Metal housing and control for reflux regulator
C_2.	Tungsten rod, 12 AWG
C_3.	Ground-glass stopper
C_4.	Drop counter
C_5.	Connection to receiver D_1
C_6.	Connection to source of low pressure, D_{21}

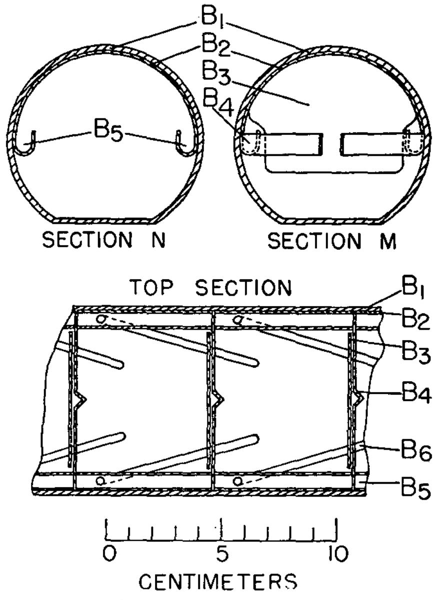

Figure 4. Cross section and top section of rectifying portion of apparatus

N and *M* are taken at middle and end, respectively, of one plate. B_1 to B_6 have significance indicated in Figure 2

In practice, the bottom side of the rectifying section is heated to vaporize a portion of the liquid. The vapor condenses on the cooler upper walls. The condensate flows to the troughs and is delivered through stainless steel tubes to the next higher plate. In this way distillate passes upward through the rectifying section to the head, where most of it is returned as reflux. The reflux returns to the bottom of the rectifying section by passing over the ridges in the lower flattened side of the glass tube. The ridges serve to maintain a small amount of liquid in each plate. At the junction between the halves, the high ridge, B_9 (Figure 2), maintains a level of liquid sufficient to cause the flow of condensate through tube B_{11} to the upper half. As the level of liquid in plate 26 is above that in plate 25, reflux returns through tube B_{10} to the lower half.

Some warping may occur during the hard-soldering and spot-welding operations involved in the construction of the internal stainless steel fittings. The effect of such warping may be minimized by constructing the fittings in relatively short lengths. Thus, for each half of fractionating section two lengths of fitting are used. The junction of the two lengths of fitting occurs at the division between two plates. Only one vapor barrier is required at this point, but both lengths of abutting fitting require liquid-tight seals in the troughs.

Figure 5 shows the receiving assembly and system for producing the low pressure. Figure 6 gives a cross section of the rectifying section and its supports.

Each half of the rectifying section is supported independently by means of a segment of an aluminum tube, E_4, which is itself supported on two Unistrut brackets, E_2, one near each end. Each length of aluminum tube is fastened at the lower end only and is free at the upper end to slide through the Transite collar, E_3, as it expands or contracts. The glass rectifying section comes in direct contact with a layer of asbestos fiber, E_6, which provides a cushion sufficiently resilient to permit expansion or contraction of the aluminum tube without excessively straining the glass.

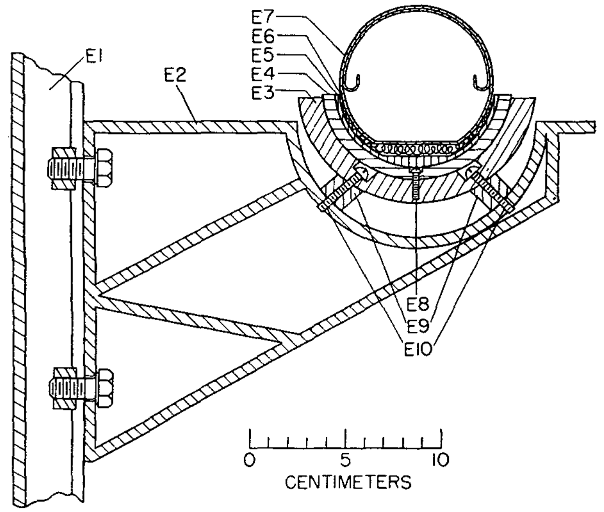

Figure 6. Cross section of support for rectifying section

E_1.	Frame, Unistrut
E_2.	Bracket, Unistrut
E_3.	Collar, Transite
E_4.	Segment of aluminum tube
E_5.	Asbestos paper
E_6.	Asbestos fiber
E_7.	Fractionating section
E_8.	Bolt fastening aluminum tube to Transite collar
E_9.	Transite blocks
E_{10}.	Bolts fastening Transite collar to bracket

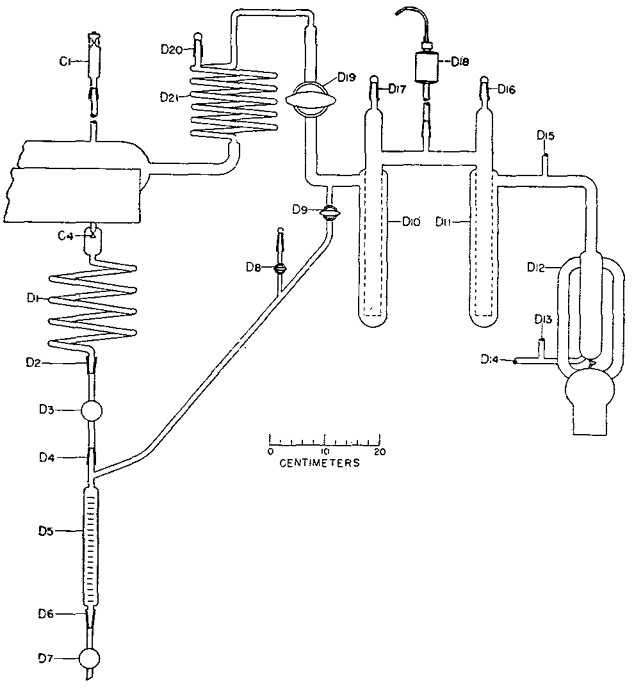

Figure 5. Receiving assembly and evacuating system

C_1.	Metal housing and control for reflux regulator
C_4.	Drop counter
D_1.	Coiled tube, 10-mm. O.D., connecting fractionating section with receiver
D_2, D_4, D_6.	Monel to glass standard-taper joints, soldered
D_3, D_7.	Diaphragm valves, Monel metal
D_5.	Receiver
D_8, D_9.	Stopcocks, 4 mm. oblique bore
D_{10}, D_{11}.	Traps, refrigerated with liquid nitrogen
D_{12}.	Mercury vapor diffusion pump
D_{13}.	Connection to gas reservoir
D_{14}.	Connection to mechanical oil pump
D_{15}.	Connection to McLeod gage
D_{16}, D_{17}.	Openings to traps, standard-taper joint, 12/30
D_{18}.	Ionization gage tube
D_{19}.	Stopcock, 15-mm. oblique bore
D_{20}.	Opening for charging and cleaning rectifying section
D_{21}.	Coiled tube, 10-mm. O.D., connecting fractionating section with evacuating system

The lower ends of both halves of the rectifying section are held firmly in position, while the upper ends are free to move. The coils between the two halves of the rectifying section, B_{10}, B_{11}, and B_{12} (Figures 2 and 3), and between the upper half of the rectifying section and receiver D_1 and source of low pressure, D_{21} (Figure 5), provide for expansion or contraction of the glass. The pot is held with a clamp on its neck and supported with a ring at the bottom.

Figure 7 shows the diagram of the electrical heating and thermocouple circuits.

The temperature is measured with single-junction copper-constantan thermocouples in the pot well, F_1, and in the liquid in plate 27, F_5, as well as on the outside between the glass and the asbestos fiber cushion at positions F_2, F_3, F_4, F_6, F_7, and F_8. Two special electrical Glascol heating mantles, H_1 and H_2, are used on the pot. Five heaters are used on the rectifying section—H_3, H_4, H_5, H_6, and H_7. The small heaters, H_3, H_5, and H_7, are

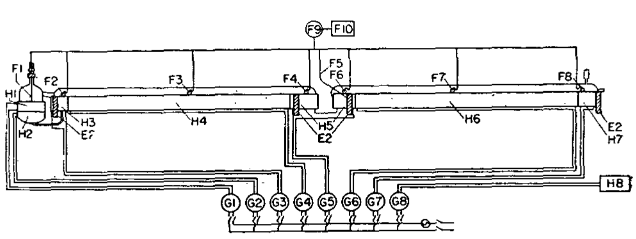

Figure 7. Diagram of electrical heating and thermoelement circuits

E_2.	Brackets supporting rectifying section (see Figure 6)
F_1.	Thermocouple to pot
F_2, F_3, F_4, F_6, F_7, F_8.	Thermocouples leading to glass on lower side of rectifying section (between E_5 and E_6 in Figure 6)
F_5.	Thermocouple to liquid in plate 27 of rectifying section (see B_{14} in Figure 3)
F_9.	Selector switch
F_{10}.	Potentiometer
H_1, H_2.	Electric heating mantles on pot
H_4, H_6.	Electric heating mantles on rectifying section
H_3, H_5, H_7.	Auxiliary electric heaters on rectifying section
H_8.	Electric heater for mercury vapor pump
G_1 to G_8.	Variable transformers, 5 amperes, 110 volts, a.c.

made of asbestos-insulated resistance wire wound directly on the aluminum tube and thermally insulated with magnesia asbestos.

A Transite box (not shown) filled with glass wool surrounds glass coils B_{10} and B_{11} (Figures 2 and 3) connecting the two halves of the rectifying section.

Figure 8 shows the design of the special heating mantles, H_4 and H_6. Figure 9 shows the entire apparatus.

OPERATING CHARACTERISTICS

The effective volume, or charging capacity, of the pot is 2.7 liters. The holdup of the entire rectifying section was found to be 0.44 liter, as determined by recovering the oil from the rectifying section after the pot had been taken to dryness. This rather high value of holdup can be reduced (to about 0.3 liter), by increasing the slope of the rectifying section so that the liquid at the upper end of each plate, which with the present slope appears to be about 2 mm. in depth, is reduced to a depth of 0.5 mm. or less.

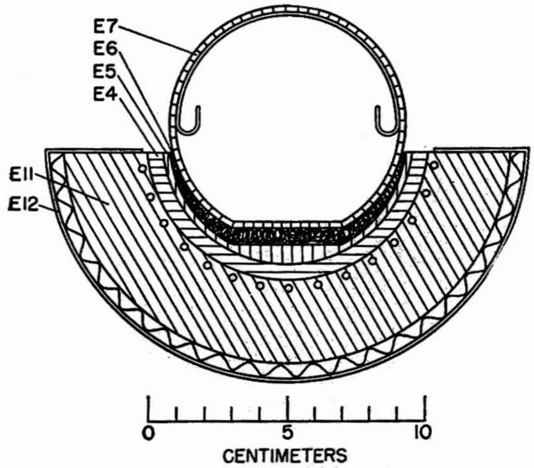

Figure 8. Cross section of electric heating mantles

E_4. Aluminum tube
E_5. Asbestos paper
E_6. Asbestos fiber
E_7. Rectifying section
E_{11}. Special glass-insulated electric heating mantle
E_{12}. Corrugated sheet asbestos covered with aluminum foil

The head of the apparatus (above plate 50) was constructed to be slightly different from the design shown in Figure 3 and had a "holdup" of 17 ml. This was determined with the apparatus in operation by opening the reflux regulator just long enough to permit material in the head to pass to the receiver, during which time (20 seconds) distillation into the head amounted to 0.5 ml. After the head had been emptied in this fashion, the throughput was determined by observing the time required for the head to fill again to the point where distillate first began to pass back over the ridge into plate 50. With *n*-eicosane in the rectifying section at a temperature of 102° C., corresponding to a vapor pressure of about 0.03 mm., the throughput was found to be 80 ml. per hour.

The time required to establish a steady state was determined by charging the apparatus (pot and rectifying section) with a test mixture consisting of a concentrate of normal paraffins from petroleum, starting the distillation process, and operating under total reflux until no change in composition occurred at the head of the apparatus. The composition in the head was followed from the refractive indices of a succession of 1-ml. samples removed at 0.5-hour intervals, starting with the onset of active distillation. A period of about 6 hours was required to establish a steady state.

PROCEDURE FOR DISTILLATION

The normal procedure for performing a distillation is as follows:

The material to be charged is introduced to the pot through opening A_2 (Figure 2). If desired, the rectifying section may also be filled through openings B_{18} (Figure 3) and D_{20} (Figure 5). Evacuation of the system is begun. When the permanent gas pressure in the apparatus has been reduced to about 1 mm., heat is applied to the pot and rectifying section and the temperatures are slowly raised. Evacuation is continued and the permanent gas pressure reduced to less than 2×10^{-5} mm.

The temperature and temperature gradient are adjusted so that the desired rate of distillation is obtained and the rate of distillation is uniform throughout the length of the rectifying

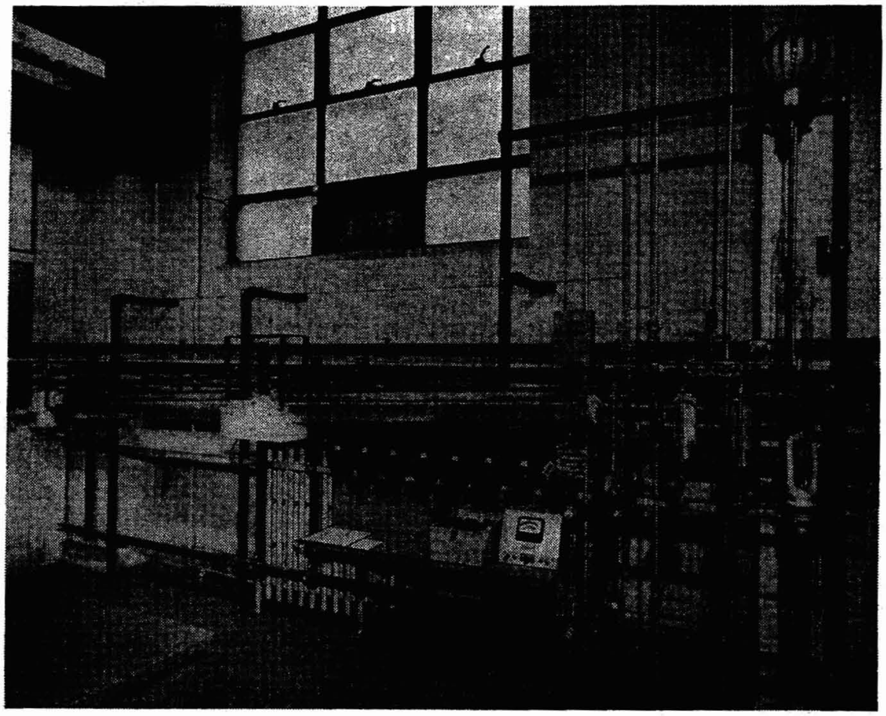

Figure 9. Assembly of 50-stage apparatus for distillation at very low pressures

section. These adjustments depend on observations of the number of drops leaving tubes B_6 in a given time at several positions along the rectifying section.

After 24 hours of normal operation in this way with total reflux, the material in the head of the apparatus (above plate 50) is allowed to flow to the receiver and is withdrawn to constitute a fraction. In this operation, with valve D_3 (Figure 5) in the open position, stopcocks D_9 and D_{19} are closed and the reflux regulator, C_1, is opened to permit the fraction to flow to the receiver, then closed. Valve D_3 is closed and stopcock D_8 is opened to the atmosphere. Valve D_7 is opened to permit withdrawal of the fraction, then closed. Stopcock D_8 is closed, stopcock D_9 opened, and the receiver evacuated. Finally, the fractionating section is reconnected to the low pressure source by opening stopcock D_{19}. The apparatus may be used for the continuous flow of distillate to the receiver, and this is preferable, but it has been found convenient to operate with total reflux, removing material from the head to constitute fractions at given intervals, as described above. When waxes or other materials which are solid at room temperature are being distilled, the receiver and connections D_1 to D_7 (Figure 5) are heated electrically. In this case it is also necessary to heat tube A_7 (Figure 2).

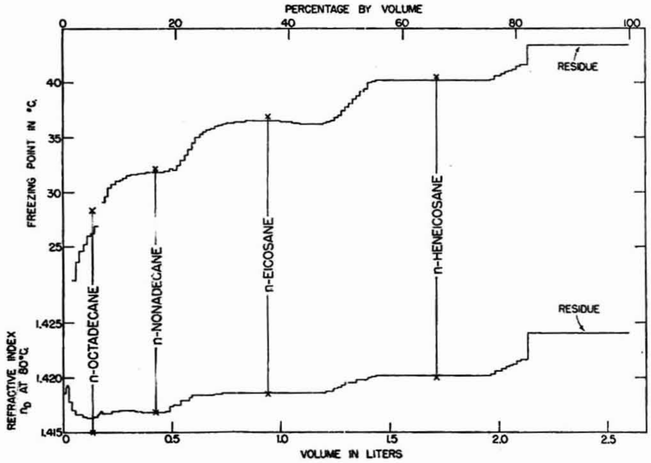

Figure 10. Results of distillation of commercial mixture of normal paraffin hydrocarbons, C_{18} to C_{22}

The ordinate scales give freezing point in ° C. and refractive index, n_D at 80° C. The scale of abscissas gives the volume of distillate in liters. The crosses give the freezing points and refractive indices of the indicated pure compounds.

The apparatus requires little attention. Usually, slight adjustments of distillation rate are made once in 24 hours, fractions are withdrawn every 12 hours, and the traps are refrigerated with liquid nitrogen once every 8 hours. The apparatus has been operated both continuously 24 hours per day, 7 days per week, and intermittently in periods of 120 hours each week. With the latter operation, because liquid remains in the plates, only several hours are required after the shutdown to reach normal operation.

RESULTS OBTAINED

Figure 10 shows the results of the distillation in this apparatus of a charge of 2.7 liters of a commercial mixture (supplied through the courtesy of the Atlantic Refining Co.) of normal paraffins near C_{20}. Figure 11 shows the results of the distillation of a charge of 2.8 liters of a concentrate of normal paraffins, C_{17} to C_{25}, separated from petroleum by molecular compound formation with urea. Both distillations were performed at a throughput of 80 ml. per hour with a reflux ratio of 55 to 1. The vapor pressure of the liquid under distillation was about 0.03 mm. of mercury.

It is apparent that the distillation referred to in Figure 10 served to separate the mixture easily into its component normal paraffin hydrocarbons, C_{18} to C_{22}. The normal paraffin hydrocarbons obtained from the distillation referred to in Figure 11 were not of very high purity, primarily because of the presence of considerable amounts of hydrocarbons other than normal paraffins in the original charge.

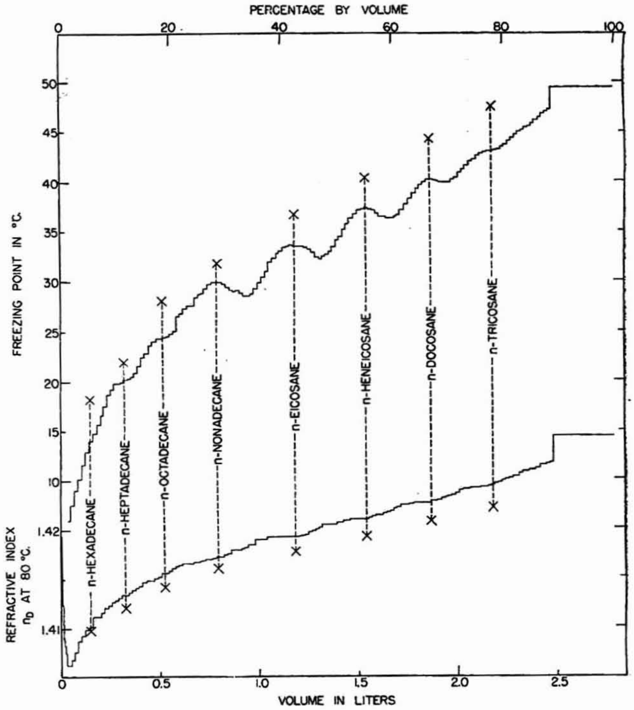

Figure 11. Results of distillation of concentrate of normal paraffins, C_{18} to C_{25}, from petroleum

The ordinate scales give freezing point in ° C, and refractive index, n_D at 80° C. The scale of abscissas gives the volume of distillate in liters. The crosses give the freezing points and refractive indices of the indicated pure compounds.

Experience on the assembly and operation of this apparatus to date indicates that it is practical to make, if needed, a 100-stage apparatus for distillation at very low pressures.

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RECEIVED for review September 13, 1954. Accepted December 7, 1954. Part of the work of the American Petroleum Institute Research Project 6 in the Petroleum Research Laboratory, Carnegie Institute of Technology. Material taken from a dissertation submitted to the Carnegie Institute of Technology in partial fulfillment of the requirements for the degree of doctor of philosophy.

[End of Symposium]

Paper Chromatographic Separation of Metal 2-Thenoyltrifluoroacetone Chelates

EUGENE W. BERG and RUSSELL T. McINTYRE

Coates Chemical Laboratories, Louisiana State University, Baton Rouge, La.

The efficient paper chromatographic separation of iron(III), copper(II), nickel(II), cobalt(II), and manganese(II) 2-thenoyltrifluoroacetone chelates was studied thoroughly to find factors influencing the separability of the various chelates. The relationship among R_f values, different adsorbers, chelate solubilities, total polarizations, and polarizabilities was determined. No single factor was found to predominate in determining the sequence of adsorption of the metal chelates on paper strips. A plausible explanation for the adsorption sequence was obtained by considering the relative solubilities, polarizations, and polarizabilities of the various chelates.

EARLIER work by the authors (*1*) showed that various mixtures of the iron(III), cobalt(II), copper(II), nickel(II), and manganese(II) 2-thenoyltrifluoroacetone chelates were resolved on paper by an ascending chromatographic technique employing a mixed solvent of benzene, methanol, and glacial acetic acid. Some difficulties experienced in finding a suitable solvent system for the separation of the components was thought to be due to the expected gross similarity of the structures of the various chelates. Each of the cobalt(II), nickel(II), manganese(II), and copper(II) ions coordinates with 2 moles of the chelating agent, whereas the iron(III) ion coordinates with 3 moles of the chelating agent. The molecular weights of the iron(III), copper(II), nickel(II), cobalt(II), and manganese(II) 2-thenoyltrifluoroacetone chelates are, respectively, 718.8, 505.5, 500.7, 500.9, and 496.9. Except for iron, the differences in weight are very small. The molecule of each chelate is predominantly organic and their structures are probably similar.

A study of this system was undertaken in order to obtain some information concerning the factors influencing the separability or R_f values of the various metal chelates.

Chromatograms of the chelate mixtures were obtained on plain paper and paper impregnated with common adsorbers whose relative adsorptive abilities generally were recognized. This approach was employed for two reasons: It was hoped that by using different adsorbers the separation of the chelate mixtures could be improved, and any observed deviation or regularity in the adsorption sequence, or R_f values, of the metal chelates might suggest the type of adsorption mechanism involved.

Assuming the solubility of the solute in the developing solvent to be the sole driving force moving the chelates up the paper, an attempt was made to find whether a relationship existed between the observed R_f values and solubilities of the metal chelates in various solvent compositions used. No direct relationship was observed between the solubilities and R_f values. This suggested that resistive forces were at least partly responsible for the observed adsorption sequence.

The resistive forces must be due to attractive forces between the solute molecules and the chromatographic medium. This suggested that the adsorption sequence might be explained if a relative measure of the total polarization and induced polarization or polarizability of each chelate were known.

A first-order approximation of the polarizability of solute molecules may be obtained from the refractive index difference between a solution and its solvent, if the measurements are made at a wave length removed from a region of absorption. Debye and Nauman (*3*) have used the equation $\mu - \mu_0 = \eta\alpha$ in light-scattering work. The μ and μ_0 are the refractive indices of the solution and solvent, respectively, η is the number of particles per cubic centimeter of solvent, and α is the polarizability of the solute molecule.

Anomalous behavior is observed where the refractive index measurements are made at a wave length within a region of absorption.

A measure of the relative dielectric constants of dilute equimolar solutions of similar solutes in an ideal solvent indicates the relative polarization of the solute molecules. The determination of relative dielectric constants usually involves the measurement of the capacitance of a cell filled first with solution and then with the solvent. The capacitance of the cell is measured at a frequency of approximately 10^6 cycles per second, because the time of relaxation of the molecules is short in comparison to the period of alternation.

Because interactions occur between solute and solvent, the polarization measured with the high frequency oscillometer is only an approximation of the total polarization.

APPARATUS

Beckman Model DU spectrophotometer with matched 1.000-cm. quartz cells.

B-S differential refractometer (Phoenix Precision Instrument Co.) with a two-compartment optical cell and a sensitivity of 3 units in the sixth decimal place for measurements of refractive index difference.

Bausch and Lomb grating monochromator with tungsten lamp.

Sargent Model V chemical oscillometer with oscillometric cell compensator and cell holder, Type A. Oscillometer frequency was approximately 5 × 10^6 cycles per second.

REAGENTS

Absolute ethyl alcohol. Bulk benzene redistilled over calcium oxide. Glacial acetic acid. Ethyl acetate, C.P.

2-Thenoyltrifluoroacetone (99% pure, molecular weight 222) was obtained from the University of California. A 10%, by weight, solution of the 2-thenoyltrifluoroacetone in 95% ethyl alcohol was used as the chelating agent.

Iron(III), copper(II), cobalt(II), manganese(II), and nickel(II) nitrates (reagent grade) were used as the source of metal ions without further purification. Aqueous solutions were made up to 1%, by weight, of the metal ion.

Whatman No. 1 filter paper was used as the chromatographic medium. Sodium chloride–impregnated paper was prepared by dipping strips of filter paper into a 10% aqueous sodium chloride solution and drying the paper in air.

Alumina-impregnated paper was prepared by the method of Datta *et al.* (*2*).

Silicic acid–impregnated paper was prepared by the method described by Kirchner and Keller (*4*).

Starch-impregnated paper was prepared by dipping the paper into a suspension of starch (40 grams of soluble starch in 1 liter of distilled water) and then drying in air.

Before use, the filter paper strips, both plain and impregnated, were stored in a vacuum desiccator over calcium chloride.

Sodium sulfide, C.P., was prepared as a 1% aqueous solution to be used as a spray reagent for the detection of cobalt.

Dimethylglyoxime was dissolved in 95% ethyl alcohol to make a 1% solution of the reagent for the detection of nickel.

Benzidine reagent for the detection of manganese was prepared by dissolving 0.05 gram of benzidine hydrochloride in 10 ml. of glacial acetic acid, diluting to 100 ml. with distilled water, and filtering.

Table I. R_f Values of Metal 2-Thenoyltrifluoroacetone Chelates in Various Solvents

Benzene-Methanol-Acetic Acid (Vol. Ratio) Solvent	Type of Paper	R_f Values of Metal Chelates				
		Fe	Cu	Ni	Co	Mn
98:0:2	Plain	0.95	0.93	0.00	0.23	0.00
	NaCl	0.98	0.94	0.00	0.05	0.00
	Starch	0.99	0.99	0.50	..	0.00
	Silicic acid	0.90	0.88	0.00	0.08	0.00
	Al_2O_3	0.91	0.58	0.00	0.01	0.00
93:5:2	Plain	0.92	0.92	0.89	0.45	0.14
	NaCl	0.94	0.94	0.02	0.27	0.04
	Starch	0.93	0.93	0.93	..	0.05
	Silicic acid	0.91	0.91	0.04	0.16	0.00
	Al_2O_3	0.91	0.89	0.00	0.00	0.00
88:10:2	Plain	0.97	0.96	0.91	0.52	0.32
	NaCl	0.95	0.92	0.55	0.26	0.08
	Starch	0.94	0.93	0.98	..	0.14
	Silicic acid	0.90	0.92	0.89	0.13	0.00
	Al_2O_3	0.93	0.92	0.00	0.00	0.00
78:20:2	Plain	0.97	0.96	0.92	0.53	0.38
	NaCl	0.95	0.94	0.88	0.39	0.30
	Starch	1.0	1.0	0.94	..	0.22
	Silicic acid	0.93	0.92	0.93	0.32	0.21
	Al_2O_3	0.93	0.92	0.49	0.00	0.00
68:30:2	Plain	0.96	0.96	0.95	0.59	0.53
	NaCl	0.96	0.94	0.90	0.54	0.49
	Starch	0.99	1.0	0.93	..	0.36
	Silicic acid	0.97	0.97	0.97	0.52	0.41
	Al_2O_3	0.90	0.94	0.70	0.09	0.00

PROCEDURE

Pure metal chelates were prepared by adding an excess of 1% aqueous solutions of the metal ions to a hot, aqueous, sodium acetate–buffered (pH 7.5) solution of 2-thenoyltrifluoroacetone. The precipitated metal chelate was filtered, washed with hot water, dried in air, and then dissolved in a minimum of ethyl alcohol. The alcoholic solution was centrifuged and the supernatant liquid decanted slowly into hot water with constant stirring to reprecipitate the chelate. The fresh precipitate was filtered, washed with hot water, and dried in vacuo over calcium chloride. The iron chelate had a tendency to form a colloidal suspension when added to hot water, unless the water contained sodium acetate. Even after recrystallization the iron chelate contained a small quantity of hydrous oxides. To remove these impurities the chelate was dissolved in ethyl alcohol and centrifuged. The alcoholic solution of the pure iron chelate was then evaporated to dryness in vacuo; the residue was stored in vacuo over calcium chloride.

The chelates prepared in the above manner were assumed to be pure compounds.

Strips of Whatman No. 1 filter paper, plain and adsorbent-impregnated, were spotted successively with 1-μl. portions of methyl isopropyl ketone solutions of each chelate to form a mixture. The diameter of the final spot was approximately 5 mm. The spotted paper strips were then hung in a closed glass chamber containing the solvent mixture to be used in the development of the chromatogram. The walls of the chamber were lined with filter paper soaked with the solvent to saturate the enclosure with vapor rapidly. One hour was permitted for equilibrium to be established between the vapor and liquid phases and then the strips were placed in contact with the developing solvent. Development was generally complete in 2 hours, when the solvent front had moved approximately 25 cm.

The positions of the manganese(II), cobalt(II), and nickel(II) chelates were determined successively as follows: Manganese was detected by streaking the paper with 0.05*N* sodium hydroxide and then with benzidine solution, a blue color indicated the presence of manganese. Cobalt was detected as the black cobalt(II) sulfide which was formed when the chromatogram was sprayed with sodium sulfide solution. The position of the nickel chelate was detected by spraying the chromatogram with a 1% solution of dimethylglyoxime in alcohol containing a trace of ammonia.

The intense colors of the iron(III) and copper(II) chelates permitted the visual observation of their positions on the developed chromatogram.

The solubility of each of the metal chelates was determined in solvent systems (benzene, methanol, and acetic acid) which had the same compositions as those used in developing the chromatograms. The dry pure metal chelate was added to several milliliters of the solvent mixture until the solution appeared saturated. An excess of the solid was then added, the test tube was stoppered, and the contents were shaken repeatedly for an hour. After centrifuging, an aliquot of the centrifugate was pipetted into a previously weighed vial. The solvent was evaporated in vacuo and the vial and residue were weighed. The solubility data were checked twice at 28° C.

Solutions of each chelate in absolute ethyl alcohol—0.01000, 0.02000, 0.03000, and 0.04000*M*—were prepared by weighing the calculated amount of the pure dry chelate into a glass vial. The weighed chelate was dissolved in a small amount of absolute ethyl alcohol, quantitatively transferred to a volumetric flask, and diluted to volume with absolute ethyl alcohol. In a similar manner 0.01000*M* solutions of each of the chelates were prepared in C.P. ethyl acetate. The standard solutions thus prepared were used in measuring the refractive index difference and the relative dielectric constant.

RESULTS

Certain ratios of benzene, methanol, and acetic acid were found to be effective in resolving a mixture of the metal chelates. Table I gives the relationship between the R_f values of the metal chelates, on plain and impregnated papers, in solvent systems in which the ratio of benzene and methanol was varied. The R_f values given are the averages of three or more determinations.

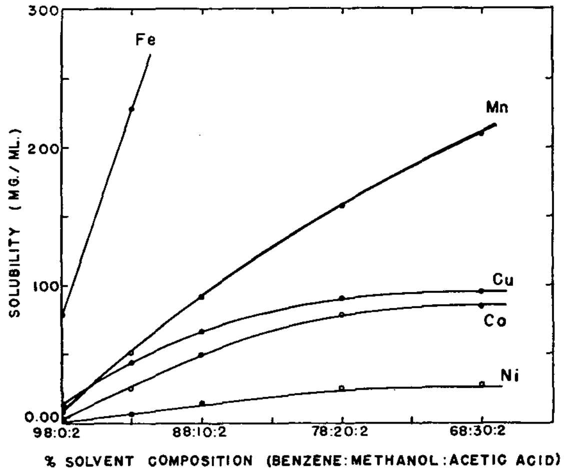

Figure 1. Relation of solubility of metal 2-thenoyltrifluoroacetone chelates to solvent composition at 28° C.

It was observed that an increase in the polarity of the solvent (an increase in methanol concentration) resulted in a larger R_f value for each chelate. In the presence of impregnated substances generally considered to be stronger adsorbents than cellulose, such as silicic acid and alumina, the migration of the chelate was less than on plain filter paper. The R_f values on plain and adsorbent-impregnated papers approached the same limiting value as the polarity of the solvent was increased.

Figure 1 shows the solubilities of the individual metal chelates as a function of the methanol concentration of the mixed solvent used in the development of the chromatogram. The order of solubility of the chelates in a solvent mixture was not in the same sequence as the R_f values of the chelates when chromatographed with a solvent of like composition.

Spectral absorption curves obtained for approximately 0.01*M* solutions of the metal chelates in absolute ethyl alcohol are shown in Figure 2. The data were collected using a Beckman Model DU spectrophotometer equipped with matched 1.000-cm. quartz cells. The blank was absolute ethyl alcohol.

Comparison of the spectral absorption curves indicated that refractive index measurements could be made on solutions of the iron(III), cobalt(II), and manganese(II) chelates at a wave length of 650 mμ because this wave length was outside a region of absorption. A longer wave length was desirable, but refractive index measurements could not be made at longer wave lengths because the visual detection of the light source was too insensitive.

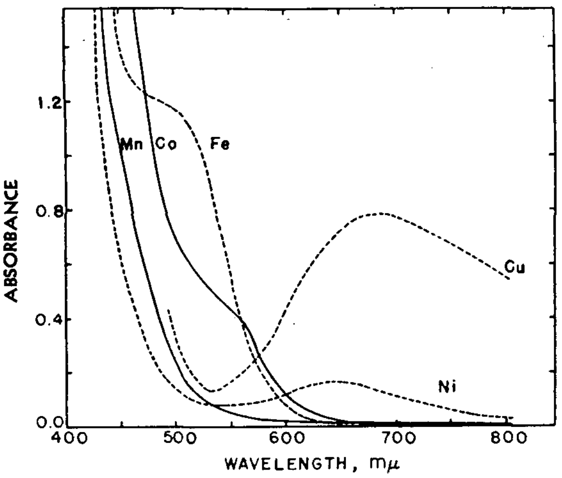

Figure 2. Spectral absorption curves of metal 2-thenoyltrifluoroacetone chelates

The low value for the absorbance of the nickel(II) chelate prompted an evaluation of the wave-length dependency of the refractive index for solutions of the nickel(II) chelate. It was observed that the rate of change of refractive index difference with wave length around 650 mμ was slight, whereas as the region of maximum absorption was approached (500 mμ and below), the refractive index differences increased greatly, indicating a region of anomalous behavior. On the basis of these observations the refractive index measurements of the nickel(II) chelate were compared with the iron(III), cobalt(II), and manganese(II) chelates at a wave length of 650 mμ.

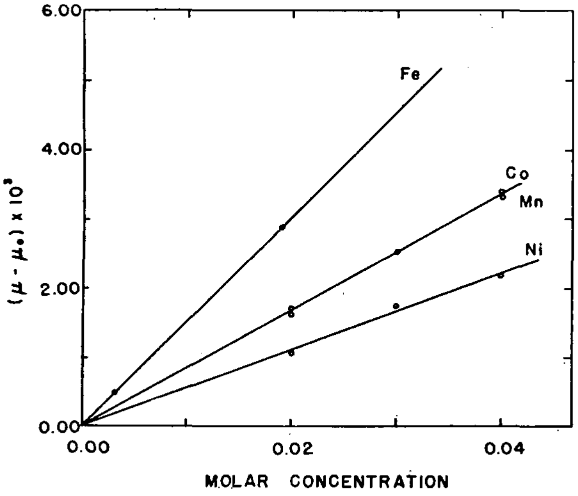

Figure 3. Refractive index difference between absolute ethyl alcohol and metal 2-thenoyltrifluoroacetone chelates in absolute ethyl alcohol

The difference in refractive index between absolute ethyl alcohol and various concentrations of the metal 2-thenoyltrifluoroacetone chelates in absolute ethyl alcohol was determined on the differential refractometer at a temperature of 28° C. The results of these measurements are plotted graphically in Figure 3.

The relative dielectric constants for the absolute ethyl alcohol and equimolar solutions of the various metal chelates in absolute ethyl alcohol were determined at 25° C. with the high frequency oscillometer. The relative capacitance of the oscillometer cell filled with pure solvent and each solution was determined several times and the results are reported as an average in Table II.

Similar data were collected for 0.01000M solutions of the metal chelates in ethyl acetate (Table III).

DISCUSSION

The observed R_f values for the metal chelates on various types of adsorbers and with different compositions of developing solvent were, with few exceptions, in the following order: iron, copper > nickel > cobalt > manganese.

The order of the solubilities of the various metal chelates in the same compositions of developing solvent used for determining the R_f values was: iron > manganese > copper > cobalt > nickel.

A comparison of the adsorption sequence and solubilities of the metal chelates in the same solvent compositions showed no direct relationship between relative solubility and R_f values except for the iron(III) chelate.

Relative polarizabilities of the various metal chelates calculated from refractive index measurements and the expression $\mu - \mu_0 = \eta\alpha$ resulted in the following order: iron > manganese, cobalt > nickel.

Table II. Relative Capacitance of Oscillometer Cell Containing 0.01000M Solutions of Metal Chelates in Absolute Ethyl Alcohol at 25° C.

Metal Chelate	Relative Capacitance of Oscillometer Cell
Fe	>32,000
Mn	31,021
Ni	30,960
Co	30,948
Cu	30,830
Ethyl alcohol	30,767

Table III. Relative Capacitance of Oscillometer Cell Containing 0.0100M Solutions of Metal Chelates in Ethyl Acetate at 25° C.

Metal Chelate	Relative Capacitance of Oscillometer Cell
Mn	13,981
Fe	13,969
Co	13,945
Ni	13,941
Cu	13,937
Ethyl acetate	13,796

The relative total polarizations measured for the metal chelates in absolute ethyl alcohol were: iron > manganese > nickel > cobalt > copper, and in ethyl acetate were: manganese > iron > cobalt > nickel > copper. These two sequences were arrived at by assuming that the relative dielectric constants, and thus the relative polarizations of the solute molecules, of the chelate solutions were directly proportional to the measured relative capacities of the cell.

Comparison of the polarizabilities and total polarization of the metal chelates to their R_f values indicated that no single factor was responsible for the observed adsorption sequence. However, if due consideration was given to the relative values of polarizabilities, solubilities, and total polarizations of the metal chelates the adsorption sequence could be explained in a qualitative manner. For example, the R_f value of the manganese(II) chelate was small but its relative solubility was large. In order to account for this apparent discrepancy, it was necessary to assume that some factor was responsible for retarding the migration of the manganese(II) chelate through the adsorption medium. In general, the greater the polarizability or polarization of a particle the more strongly it is adsorbed. The observed relative polarizability and polarization of the manganese are large. This could possibly account for the small R_f value for the manganese(II) chelate.

Similar comparisons of the relative polarizabilities, solubilities, and polarizations of the various metal chelates will account for the observed adsorption sequence in every instance except one.

The relative R_f value of the iron(III) chelate is large, although this chelate has the greatest polarizability and one of the greatest polarizations of the group. The solubility of the iron(III) chelate is, however, five- to sixfold greater than that of any other chelate. The relatively large solubility would probably be sufficient to overcome the differences in adsorbability of the various metal chelates due to the relatively small differences in polarizabilities and polarizations.

The data presented suggest that in some chromatographic systems there are numerous factors that affect the adsorption sequence.

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Received for review July 6, 1954. Accepted September 9, 1954.

Comprehensive Analysis of Sodium Alkyl Aryl Sulfonate Detergents

F. T. WEISS, A. E. O'DONNELL, R. J. SHREVE, and E. D. PETERS
Shell Development Co., Emeryville, Calif.

Satisfactory methods have not been available for the complete analysis of sodium alkyl aryl sulfonate detergents, which are of current commercial importance. As the result of this investigation, which involved a survey of available techniques, a suitable modification of the ASTM D 855/46T method for the analysis of petroleum sulfonates was developed. Reliable values are obtained for the content of sodium sulfonate, inorganic salts, neutral oil, sodium carboxylate, free acid or alkali, water, and for the average combining weight of the sodium sulfonates. The method is applicable to sodium alkyl aryl sulfonate detergents containing organic and inorganic builders and, with minor modifications, can also be applied to sodium alkyl sulfate detergents. The method has been successfully used to analyze a wide variety and large number of experimental and commercial alkyl aryl sulfonate detergents.

Large quantities of sulfonate detergents are currently being produced by sulfonation of alkylated aromatics, the most common aromatic base being dodecylbenzene. Certain commercial detergents consist essentially of the dried neutralized sulfonation product and thus contain a large proportion of sodium sulfate from the sulfuric acid used in the sulfonation. Most commercial detergents, however, are made by replacing part of the sodium sulfate with inorganic salt builder for the purpose of increasing the effectiveness of the detergent. Organic additives, such as sodium carboxymethylcellulose or nonionic detergents, are also sometimes added. Mixed sodium alkyl sulfate and sodium alkyl aryl sulfonate detergents have been prepared.

For satisfactory comprehensive analysis of experimental and commercial sodium alkyl aryl sulfonate detergents, determination of the following components has been found necessary: sodium sulfonate, inorganic salts, neutral oil, free acid or alkali, water, and sodium carboxylates resulting from minor oxidative side reactions during sulfonation. In addition, determination of the average combining weight of the sodium sulfonates has been of value. Determination and identification of organic additives may be required.

A number of methods for the analysis of detergent mixtures containing sulfonates have been proposed in the literature. The majority of these methods fall under the following two classifications:

The more or less complete methods, which generally involve separation and gravimetric determination of components. This group of methods has been examined for the purpose of providing the basis for the desired comprehensive analysis.

Methods for the specific determination of the sulfonate group, generally through the use of amine salts, such as benzidine hydrochloride (*5, 17*), *p*-toluidine hydrochloride (*20*), methylene blue (*16*), and cetyl pyridinium bromide (*8*). These methods generally give a value only for the equivalents of surface active sulfonate present and provide no information concerning the other components of the detergent mixtures. Because of their rapid and simple nature, these methods are useful for process control but are not suitable for complete analysis.

Early work in the development of separation methods for the analysis of detergent mixtures was done by Hart (*10–14*), who published procedures for determination of the number of equivalents of sulfonate (or alkyl sulfate) and for inorganic salt content of detergent mixtures. Berkowitz and Bernstein (*4*) proposed a method for direct determination of inorganic salts, neutral oil, and sodium carboxylate in soap–synthetic detergent mixtures, in which synthetic detergent was calculated by difference. The ASTM method for the analysis of sulfonated and sulfated oils (*3*) utilizes several of the procedures developed by Hart and by Berkowitz and Bernstein. Considerable effort has been applied to the development of methods for the analysis of oil-soluble sodium petroleum sulfonates, materials of higher average combining weight than the detergent sulfonates. Brooks, Peters, and Lykken (*6*) have reviewed earlier work and presented an extraction-adsorption method for the analysis of petroleum sulfonates. Another method for the analysis of sodium petroleum sulfonates (*1*) is reliable and is well established in the petroleum industry. Of the available methods which appeared applicable to the analysis of sulfonate detergents, the methods used for the analysis of petroleum sulfonates were considered more promising because of their more comprehensive nature and more direct determination of sodium sulfonate.

Investigation of the behavior of several typical sodium alkyl aryl sulfonate detergents in the two petroleum sulfonate methods revealed that the extraction-adsorption procedure was generally unsatisfactory with these materials. Excessive loss of some sulfonic acids to the aqueous phase during partition of the materials between chloroform and hydrochloric acid occurred. In addition, evidence of decomposition of sulfonic acids upon evaporation of the chloroform phase was found, although this reaction presumably does not occur to an appreciable extent with the more highly substituted petroleum sulfonic acids. On the other hand, the ASTM method was found to be much more satisfactory in its application to the sulfonate detergents.

In the application of the ASTM method (*1*) to commercial and experimental sodium alkyl aryl sulfonate detergents, it became apparent that a number of modifications were desirable. The high inorganic salt content of the detergent samples made it necessary to increase the proportion of water used in dissolving the sample prior to extraction with petroleum ether. Because of the hygroscopic nature of the sodium alkyl aryl sulfonates, it is necessary to determine and correct for the small but appreciable amounts of water in the residues containing sulfonate. Since small amounts of sodium carbonate are always found in the deoiled desalted alcohol phase, a correction for sodium carbonate was introduced. If sodium chloride is present in the sample, some sodium chloride will be found in the deoiled desalted alcohol phase and consequently a correction for this salt is included in the method. The Fischer method has been chosen for water determination because this is more rapid and convenient than the distillation method. Although the detergent method as finally adopted is similar to the ASTM D 855 method, a complete description is given of the importance and frequency of the differences in details between the two methods.

APPARATUS AND PROCEDURE

Apparatus of the type described by the ASTM (*2*) was used.

Determination of Free Basicity or Acidity. Introduce into a 250-ml. beaker sufficient sample to contain from 3 to 5 grams of sodium alkyl aryl sulfonate, weighed to the nearest centigram. Add 125 ml. of distilled water and 50 ml. of isopropyl alcohol (99%), and warm gently on a steam bath with stirring to dissolve the sample. Cool the solution to room temperature, add a few drops of phenolphthalein indicator, and neutralize with 0.1*N* sulfuric acid or 0.1*N* sodium hydroxide.

Separation of Neutral Oil from Inorganic Salts and Sodium Sulfonates. Quantitatively transfer the neutralized solution to a 500-ml. separatory funnel, rinsing the beaker and stirring rod with 10 ml. of distilled water followed by 10 ml. of isopropyl alcohol. Rinse the sampling beaker with 30 ml. of petroleum ether and use this portion of ether to extract the combined alcoholic solution; retain the interface cuff with the ether phase. Extract the alcoholic phase five more times with 30-ml. portions of petroleum ether, using two 250-ml. separatory funnels and collecting the petroleum ether extracts in the 500-ml. separatory funnel. Drain the oil-free alcoholic sulfonate solution into a 500-ml. mixing cylinder. Rinse each 250-ml. separatory funnel with two 10-ml. portions of isopropyl alcohol (50%) and add to the mixing cylinder. Wash the combined ether extracts with 50 ml. of isopropyl alcohol (50%); add the wash to the mixing cylinder.

Determination of Neutral Oil. Filter the combined ether extracts through a small plug of cotton, placed in the vortex of a filter funnel, into a tared inverted-rim beaker. Wash the separatory funnel and filter with 20 to 30 ml. of petroleum ether, adding the washings to the inverted-rim beaker. Evaporate the ether solution to dryness on a steam bath, adding small portions of isopropyl alcohol to aid in removing water. Heat for 20 minutes after the disappearance of the odor of alcohol. Cool to room temperature and bring to constant weight in a vacuum desiccator at room temperature at a pressure under 3 mm.

Determination of Sodium Sulfonate in Neutral Oil. Weigh a 1- to 2-gram sample of the oil residue into a tared, ignited platinum dish. If only a small amount of oil is obtained, suspend the entire oil residue in isopropyl alcohol and quantitatively transfer to the platinum dish, using a medicine dropper. Remove the solvent by evaporation on a steam bath. Heat with a Bunsen burner until the contents ignite and burn, then maintain at such a temperature that only ash and carbon remain after burning ceases. Ignite over a burner, or in a muffle furnace at 500° to 550° C., until the oxidation of carbon is complete. Cool and add 3 to 4 drops of sulfuric acid (36*N*). Fume off the sulfuric acid over a burner in a hood. Add another 3 to 4 drops of sulfuric acid (36*N*) and again remove the acid by fuming. When fuming ceases, heat in a muffle furnace at 800° to 1000° C. to constant weight. Cool the dish in a desiccator after removing from the muffle and prior to weighing.

Determination of Deoiled Total Solids. Measure the volume of the oil-free alcohol solution of inorganic salts and sodium sulfonates and pipet a 25-ml. aliquot into a tared 150-ml. beaker, evaporate to dryness on a steam bath, and dry in an atmospheric oven at 120° C. for 8 hours. If alkyl sulfates are present, add 0.2 ml. of 0.1*N* sodium carbonate solution to the aliquot and dry in an oven at 75° C. Cool the dry residue in a desiccator after removing from the oven and prior to weighing.

Determine the water content of the residue, using the major amount of the residue.

Separation of Sodium Sulfonates and Carboxylates from Inorganic Salts. Add 65 ml. of isopropyl alcohol to the mixing cylinder and warm in a water bath at 40° to 50° C. Add 18 grams of anhydrous sodium carbonate per 100 ml. of alcohol solution remaining in the mixing cylinder. Admit only a few grains to the mixing cylinder at first and allow the dissolved petroleum ether to evaporate slowly. When danger of boiling is past, add the remainder of the carbonate and shake the mixture vigorously. Allow to stand for a few minutes and repeat the shaking. Allow the phases to separate, swirling the solution to dislodge any solid particles clinging to the upper part of the mixing cylinder. Replace the mixing cylinder in the water bath at 40° to 50° C. until separation into two layers is complete. Cool the mixing cylinder to room temperature (3 to 4 hours) and measure the volume of the alcohol phase.

Determination of Sodium Sulfonates and Carboxylates. Pipet a 75-ml. aliquot of the alcohol phase into a tared 250-ml. beaker and evaporate it to dryness on a steam bath. Dry for 8 hours in an atmospheric oven at 120° C. If sodium alkyl sulfates are present, add 10-ml. portions of water and evaporate to dryness several times to remove alcohol and dry in an oven at 75° C. Cool the residue in a desiccator after removing from the oven and prior to weighing.

Determine the water content of the residue using approximately one third of the residue.

Determination of Sodium Carboxylates. Weigh a portion of the sodium sulfonates and carboxylates residue into a beaker; dissolve in a 100-ml. portion of water and transfer the solution quantitatively to a 500-ml. separatory funnel. Wash out the beaker with two 75-ml. portions of water and add the washings to the funnel. Add 50 ml. of 12*N* hydrochloric acid to the funnel and shake vigorously. Allow the solution to cool and then extract the liberated acids with two 100-ml. portions of ethyl ether. Combine the ether extracts in a 500-ml. separatory funnel. Wash the combined ether extracts until free of strong acid by shaking with two or more 50-ml. portions of aqueous sodium sulfate (20%) containing methyl orange indicator. Discard the salt washings.

Drain off as much water as possible from the washed ether extract. Lay the separatory funnel on its side and introduce about 10 grams of anhydrous sodium sulfate. Shake the mixture vigorously for 3 to 4 minutes, venting frequently. Filter the ether solution, using a small plug of cotton in the vortex of a filter funnel, into a 250-ml. Erlenmeyer flask. Rinse the funnel and filter with 20 ml. of ethyl ether, add the rinsings to the main ether solution, and remove all the ether by evaporation on a steam bath.

Determine the acid content of the residue as follows: Add 4 or 5 drops of phenolphthalein indicator and 50 ml. of 50% isopropyl alcohol, previously neutralized. Warm if necessary to dissolve the sample and titrate with standard 0.1*N* sodium hydroxide solution to a definite pink color.

Determination of the Average Combining Weight of the Sodium Sulfonates. Weigh approximately one third of the dried sodium sulfonates and carboxylates residue into a tared, ignited platinum dish. Ignite and ash the contents of the dish using the procedure given earlier.

Determination of Inorganic Salt Contaminants in the Deoiled, Desalted Alcoholic Sulfonate Solution. SODIUM CARBONATE. Pipet 25 ml. of the deoiled, desalted alcoholic sulfonate solution into a 150-ml. beaker containing 50 ml. of distilled water and a few drops of phenolphthalein indicator. Heat to a gentle boil and titrate with standard 0.1*N* sulfuric acid to the disappearance of the pink color. Repeat until the pink color fails to return on additional boiling.

SODIUM CHLORIDE. If sodium chloride is present in the original sample, determine the sodium chloride content of a 25-ml. aliquot of the isopropyl alcohol solution by amperometric titration (*19*) or, with somewhat lowered accuracy, by the Volhard method.

Determination of the Water Content of the Sample. Determine the water content of dried detergent samples by the Fischer method. Introduce 10 ml. of anhydrous methanol and 10 ml. of anhydrous pyridine into a clean 100-ml. volumetric flask, avoiding wetting the neck of the flask. Titrate with Fischer reagent until the straw-yellow color changes to an orange-red which remains after shaking the stoppered flask; view the color by transmitted light. Avoid wetting the neck of the flask when shaking. Weigh the stoppered flask to the nearest centigram and then insert a piece of paper, rolled into a tube, part-way down the neck of the flask and add 2 to 3 grams of sample. Remove the paper and reweigh the stoppered flask. Titrate with Fischer reagent to the orange-red color of a reference blank.

In Figure 1 is presented a diagram of the entire procedure for the analysis of sodium alkyl aryl sulfonate detergents.

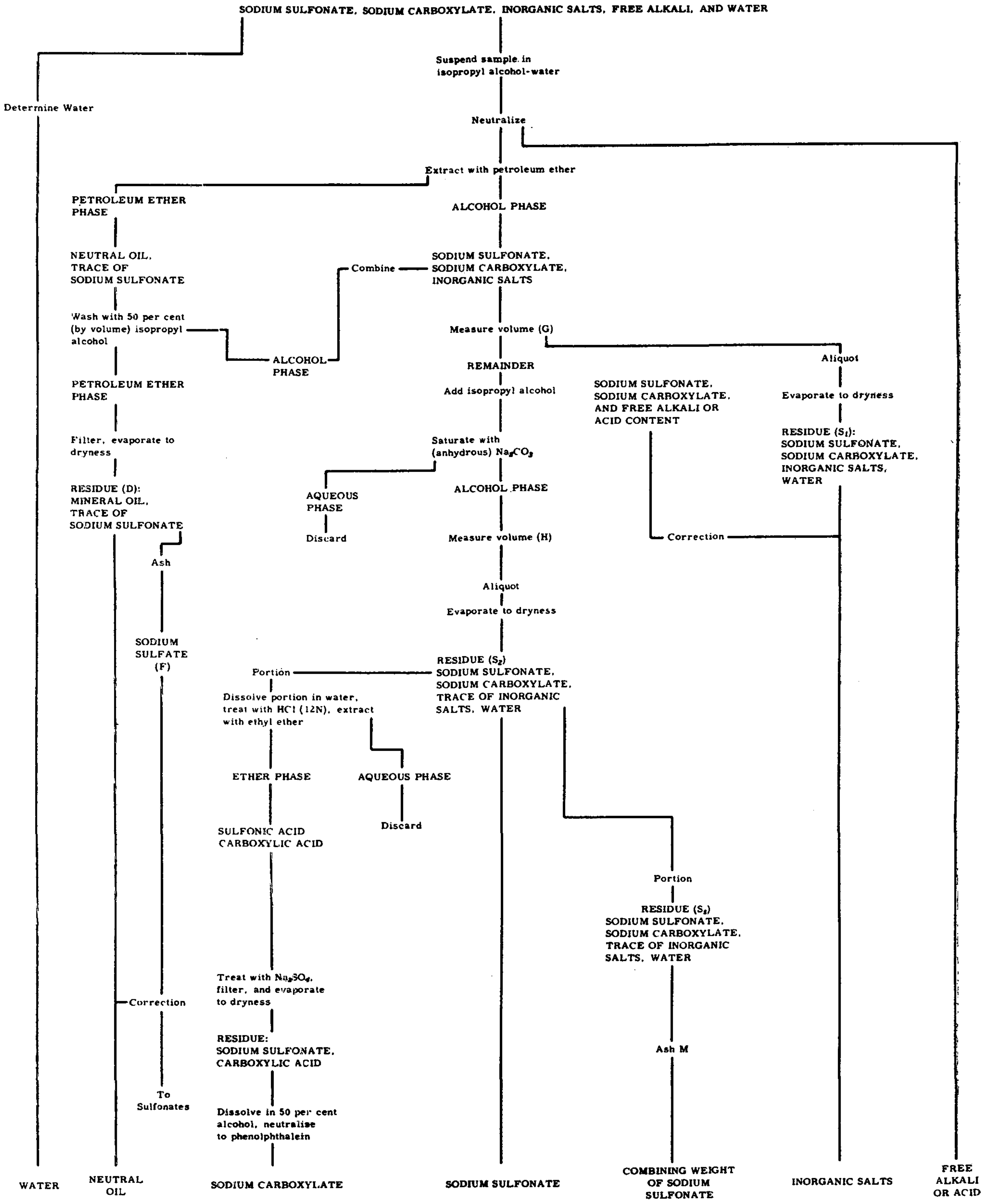

Figure 1. Outline of method of analysis of sodium alkyl aryl sulfonate detergents

CALCULATIONS

$$\text{Dry weight of } S_2 \text{ residue} = \frac{(S_2)(100 - V)}{(100)} \qquad (1)$$

Weight of sodium sulfonate in S_2 and S_3 residues:

$$W = (0.053)(N)(O) + (0.0585)(R) + \frac{(0.298)(K)(L)(S_2)}{(3)(J)} + 0.0011 \qquad (2)$$

$$S_2\,(\text{corrected}) = [S_2\,(\text{dry})] - 3\,(W) \qquad (3)$$

$$S_3\,(\text{corrected}) = \frac{(S_3)}{(S_2)}\,[S_2\,(\text{corrected})] \qquad (4)$$

Omit the term (+0.0011) in Equation 2 unless sodium carbonate solution was added to stabilize sodium alkyl sulfates. The factors (0.053), (0.0585), and (0.298) in Equation 2 are the weights in grams per milliequivalent of sodium carbonate, sodium chloride, and sodium carboxylate, respectively. The last factor is an assumed value and when known the accurate value should be used. The number 3 in the denominator is an aliquot factor.

Corrected sulfate ash:

$$M\,(\text{corrected}) = M - \frac{(3)(S_3)}{(S_2)}\,(0.071)\left[(N)(O) + (R) + \frac{(K)(L)(S_2)}{(3)(J)} + 0.02\right] \qquad (5)$$

Omit the term of +0.02 in Equation 5 unless sodium carbonate solution was added to stabilize sodium alkyl sulfates.

Average combining weight of sodium sulfonates:

$$X = \frac{(71)\,[S_3\,(\text{corrected})]}{M\,(\text{corrected})} \qquad (6)$$

$$\text{Neutral oil content, per cent,} = \left[\frac{(100)(D)}{(A)}\right] - \left[\frac{(100)(F)(X)}{(71)(A)}\right]\left[\frac{D}{E}\right] \qquad (7)$$

Sodium sulfonate content, per cent, =

$$\left[\frac{(100)\,[S_2\,(\text{corrected})]}{(A)}\right]\left[\frac{G}{G - 25}\right]\left[\frac{H}{75}\right] + \left[\frac{(100)(F)(X)}{(71)(A)}\right]\left[\frac{D}{E}\right] \qquad (8)$$

Basicity as sodium hydroxide:

$$Y = \frac{(40)(B)(C)}{(10)(A)} \qquad (9)$$

Free acidity as sulfuric acid:

$$Y = \frac{(49)(B)(C)}{(10)(A)} \qquad (10)$$

Sodium carboxylate content:

$$Z = \left[\frac{(100)(0.298)(K)(L)}{(A)}\right]\left[\frac{G}{G - 25}\right]\left[\frac{H}{75}\right]\left[\frac{S_2}{J}\right] \qquad (11)$$

Inorganic salts:

$$S_1\,(\text{dry}) = \frac{(S_1)(100 - U)}{(100)} \qquad (12)$$

$$\text{Inorganic salt content, per cent,} = \left[\frac{(100)\,[S_1\,(\text{dry})]}{(A)}\right]\left[\frac{G}{25}\right] - \left[\frac{(100)\,[S_2\,(\text{corrected})]}{(A)}\right]\left[\frac{G}{G - 25}\right]\left[\frac{H}{75}\right] - (Z) - \left[\frac{(71)(B)(C)}{(10)(A)}\right] \qquad (13)$$

Nomenclature

A = weight of original sample taken for analysis, grams
B = volume of sulfuric acid or sodium hydroxide required to neutralize free alkali or acid, milliliters
C = sodium hydroxide or sulfuric acid solution, N
D = weight of oil residue, grams
E = weight of oil residue taken for ashing, grams
F = weight of sodium sulfate ash from oil residue, grams
G = volume of alcoholic deoiled salt and sulfonate solution, milliliters
H = volume of salted-out alcohol layer, milliliters
J = weight of portion of sodium sulfonate and carboxylate residue taken for carboxylate determination, grams
K = volume of sodium hydroxide required to neutralize the carboxylic acids, milliliters
L = sodium hydroxide used to neutralize the carboxylic acids, N
M = weight of sulfate ash from deoiled, desalted sulfonate and carboxylate residue taken for combining weight determination, grams
N = volume of sulfuric acid required to neutralize sodium carbonate contaminants, milliliters
O = sulfuric acid used to neutralize sodium carbonate contaminants, N
R = milliequivalents of chloride determined in 25 ml. of the salted-out alcohol phase
S_1 = weight of residue from determination of deoiled total solids, grams
S_2 = weight of residue from determination of sodium sulfonates and sodium carboxylates, grams
S_3 = weight of deoiled, desalted sodium sulfonate and sodium carboxylate residue taken for determination of combining weight of the sodium sulfonates, grams
U = water content of S_1 residue, per cent

Table I. Results Obtained in the Analysis of Unbuilt Sodium Alkyl Aryl Sulfonate Detergents

Determination	Sodium Dodecyltoluene Sulfonate Detergent		Sodium Dodecylbenzene Sulfonate Detergents							Sodium Keryl Benzene Sulfonate	
					Sample 2						
			Sample 1		Analyst A		Analyst B	Sample 3			
Neutral oil, wt. %	0.7	0.7	1.9	2.3	1.5	1.5	1.4	3.2	3.2	0.8	0.6
Sodium sulfonate, wt. %	37.8	38.2	33.6	33.2	36.8	36.9	36.8	95.2	93.7	39.5	40.0
Inorganic salts, wt. %	59.9	59.5	63.9	63.3	59.8	59.9	59.1	0.1	0.1	55.5	55.6
Sodium carboxylate, wt. %	0.4	0.3	0.4	0.5	...	...	...	0.1	0.1	0.6	0.6
Acidity (as H_2SO_4), wt. %	...	...	...	...	0.05	0.06	0.1	...	...	0.3	0.3
Basicity (as NaOH), wt. %	0.003	0.005	0.06	0.06	...	...	...	<0.01	<0.01	...	...
Water, wt. %	1.2	1.3	1.7	1.6	2.0	2.0	2.5	1.9	1.9	3.3	3.3
Total, wt. %	100.0	99.9	101.6	101.0	100.2	100.4	99.9	100.4	99.0	100.0	100.4
Combining wt. of sodium sulfonate, grams per equiv.											
Det.	354, 361		354, 356		340, 340		336	330, 328		319, 320	
Calcd.	363		348		348		348	348		340	
Organic material in separated aq. phase, calcd. as sodium sulfonate[a], wt. % of original sample	0.36, 0.37		0.4, 0.4		0.11		...	...		0.7	
Inorganic salts in desalted alc. phase, wt. % of total solid in alc. phase											
Na_2CO_3	0.16, 0.23		0.43		0.36, 0.41		0.45	...		...	
Na_2SO_4[b]	0.01, 0.01		...		0.04, 0.04		...	...		...	

[a] Calculated from carbon content of separated aqueous phase, determined by wet oxidation (*15*) after removal of alcohol and carbonate.
[b] Amperometric. This value includes any Na_2SO_3 present in the desalted alcohol phase.

V = water content of S_2 residue, per cent
W = inorganic salt and sodium carboxylate correction for S_2 residue, grams
X = average combining weight of sodium sulfonates, grams per equivalent
Y = percentage of free basicity calculated as sodium hydroxide or free acidity calculated as sulfuric acid
Z = sodium carboxylate content of sample, per cent

RESULTS AND DISCUSSION

Analysis of Unbuilt Sodium Alkyl Aryl Sulfonate Detergents. Unbuilt detergents are defined as those containing no additives, either inorganic or organic. Consequently, the inorganic salt consists almost entirely of sodium sulfate, together occasionally with minor amounts of sodium carbonate and sodium sulfite. In Table I are presented results obtained in the analysis of several typical unbuilt sodium alkyl aryl sulfonate detergents. In addition data are given in Table II which were obtained in the analysis of sodium benzene sulfonate and sodium *p*-toluene sulfonate. The results shown in these two tables are typical of those which can be obtained on a wide range of sulfonates. A critical appraisal of the various component parts of the method as applied to unbuilt detergents is presented in the following sections.

Table II. Results Obtained in Analysis of Low Molecular Weight Sulfonates

Determination	Sodium Benzene Sulfonate (Eastman)	*p*-Toluene Sulfonic Acid (Monohydrate[a]) (Eastman)
Neutral oil, wt. %	1.0, 1.0	0.1, 0.0
Sodium benzene sulfonate, wt. %	98.4[b], 98.4[b]	...
Toluene sulfonic acid, wt. %	...	89.2[b], 89.0[b]
Inorganic salts, wt. %	0.5, 0.1	...
Sulfuric acid, wt. %	...	0.3[b], 0.6[b]
Basicity as NaOH, wt. %	Neutral to phenolphthalein	...
Water, wt. %	0.1, 0.1	9.8, 9.8
Combining wt. of sodium sulfonate, grams per equiv.		
Det.	183, 182	198, 195
Theor.	180	194
Organic material in separated aq. phase, calcd. as sodium sulfonate, wt. % of total sodium sulfonate found[c]	2.6, 2.9	0.9, 1.0
Inorganic salts in desalted alcohol phase, wt. % of total solid in alcohol phase		
Na_2CO_3	0.92, 0.98	3.88, 4.18
Na_2SO_4[d]	...	0.014, 0.015

[a] Analysis performed on neutralized sample.
[b] Corrected for sodium sulfonate in separated aqueous phase.
[c] Calculated from carbon content of separated aqueous phase, determined by wet oxidation (*15*) after removal of alcohol and carbonate.
[d] Amperometric. This value includes any Na_2SO_3 present in the desalted alcohol phase.

Neutral Oil. Recovery of hydrocarbons in this procedure was found to be from 93 to 95% in experiments made with cetane, C_{12}-benzene alkylate, and C_{12}-toluene alkylate, but as expected, was considerably less with hydrocarbons of lower molecular weight. A small amount of sodium sulfonate, generally $^1/_{200}$ of the sulfonate content of the sample, is extracted with the neutral oil. This can be measured as sodium sulfate by ashing the oil residue with sulfuric acid, and a correction applied, if necessary.

Sodium Sulfonate and Combining Weight. The three possible sources of error in this determination are loss of sulfonate to the neutral oil, loss of sulfonate to the inorganic salts, and presence of inorganic salts with the sulfonates.

The loss of sulfonate to the neutral oil is small in the analysis of sodium alkyl aryl sulfonates and can be corrected. The second source of error, loss of sulfonate to the inorganic salts, was examined by determining the carbon content of the separated aqueous phase by a wet oxidation method, after removal of alcohol and carbonate. As can be seen from the data in Table I, this source of error was of the order of 1 part in 100. Since a simple means for correcting for this loss of sulfonate is not available, none is made. The third possible error, presence of inorganic salt in the separated sodium sulfonate, is likewise small, as shown in Tables I and II, and is eliminated by determining the principal contaminant, sodium carbonate, and applying a correction. Since relatively small amounts of inorganic salts have an appreciable influence on the combining weight value, the correction for sodium carbonate is essential in this determination.

Table III. Results Obtained in the Analysis of a Sulfonate Detergent Containing Added Sodium Stearate

	Content, Wt. %	
	Known	Determined
Neutral oil	1.1	1.2
Sodium sulfonate	39.1	39.2
Inorganic salts	43.9	43.2
Sodium stearate	14.5	14.0
Combining wt. of sodium sulfonate, grams per equiv.	360	357

The water content of the dried residues containing sulfonate are generally about 1%. Since the water content is easily determined, a correction is made to avoid error from this source.

Inorganic Salts. Although the separation of sulfonate and inorganic salts is not entirely complete, the amount of sulfonate present in the aqueous phase is sufficiently small that it can be ignored when the inorganic salt content of the detergent sample is high, which is usually the case. Of the inorganic salts, sodium sulfate is quantitatively retained in the aqueous phase; sodium chloride, if present in the original sample, is partially lost to the alcohol phase; the method, however, includes a correction for this loss.

With samples having a low inorganic salt content and a high sulfonate content, the inorganic salt determination is relatively inaccurate. Not only does the presence of sulfonate in the aqueous phase give rise to error, but the indirect method of calculating inorganic salts as the difference between two large numbers—the total solids content and the sodium sulfonate content—can lead to appreciable relative error. For such detergent samples, direct means of determining the individual inorganic salt components appears the preferable approach. Such procedures as the determination of sulfate by amperometric titration with lead nitrate (*9, 19*) and the determination of carbonate and sulfite by evolution and measurement of carbon dioxide (*23*) and sulfur dioxide (*25*) appear to be promising approaches.

Sodium Carboxylate. Because sodium carboxylate is not isolated in the method but determined, as carboxylic acid, by titration with standard base, it is necessary to assume an equivalent weight (298, corresponding to $C_{18}H_{27}O_2Na$) in order to calculate its concentration. Inasmuch as the amounts of carboxylate found in the usual detergent samples are small, little error can arise from this assumption. However, when larger amounts of sodium carboxylate with an equivalent weight appreciably different from 298 are present in the sulfonate sample, use of the assumed equivalent weight value would lead to considerable error in the determination of the carboxylate content on a weight basis. In addition, since the sulfonate content is calculated from the weight of the sulfonate residue less the carboxylate content of the sulfonate residue, any error in the value for carboxylate content will be reflected in an equal but opposite error in the sulfonate content. When the equivalent weight of the carboxylate is known, however, the method is accurate even in the presence of large amounts of carboxylate as can be seen from the data in Table III.

Basicity or Acidity. The basicity value includes that due to free sodium hydroxide and to the titration of any sodium carbonate to the bicarbonate. As the total basicity is usually very low, the magnitude of the error caused by expressing the determined basicity as sodium hydroxide is negligible. If a more accurate value for the sodium hydroxide content is desired and the alkalinity is due only to sodium hydroxide and sodium carbonate, the interfering carbonate ions can be precipitated as the barium salt (*18*). When the sodium carbonate content of the sample is appreciable (above 2%) and the sodium hydroxide content is negligible, it is preferable to omit the neutralization of the sample prior to extraction and separation of components, since the sodium bicarbonate formed in the neutralization is partially decomposed during heating at 120° C. in the subsequent determination of total solids. In this case the basicity can be determined on a separate portion of the sample. Samples which are originally acidic should be neutralized prior to extraction to prevent loss of organic acids to the petroleum ether extracts and their subsequent determination as neutral oil.

WATER CONTENT. The water content of sulfonate samples is usually determined by the Fischer method. A satisfactory dispersing medium is a mixture of methanol and pyridine (1 to 1). Although inorganic salts are not appreciably soluble in this mixture, complete reaction of the water present can be obtained if the mixture is well agitated during the titration.

Sodium hydroxide, sodium carbonate, and sodium sulfite react with Fischer reagent to produce 1 mole of water per mole of basic salt (*22*). However, the concentration of these basic salts, and consequently their interference, are negligible with the usual detergent samples. When appreciable amounts of sodium hydroxide (above 0.2%), sodium carbonate (above 0.5%), or sodium sulfite (above 0.7%) are present, their concentrations must be determined to obtain a water value by the Fischer method which will be correct to 0.1%. To avoid the necessity of correcting for the reaction of these basic materials in the Fischer method, the water content of detergent samples can be determined by distillation of the sample with xylene, using Dow-Corning Antifoam A to prevent foaming followed by determination of water in the distillate by titration with Fischer reagent (*24*).

TIME REQUIREMENTS. The chief shortcoming of the method is the length of time required to perform an analysis. About 9 man-hours are required for the complete analysis of one sample with an elapsed time of approximately 32 hours. Some shortening of the elapsed time could undoubtedly be achieved by close investigation of the minimum times required for some of the lengthier steps in the procedures.

Analysis of Sodium Alkyl Aryl Sulfonate Detergents Containing Inorganic Salt Builders. The method has been found to apply satisfactorily in its general outline to detergents containing builders. However, with certain builders modification of one or more of the procedures may be necessary.

SODIUM SULFONATE AND COMBINING WEIGHT. None of the inorganic builders tested have been found in appreciable concentrations in the deoiled desalted sulfonate solution (see Table IV), with the exception of sodium carbonate, for which a correction is normally made. No significant deviation in the combining weight values were caused by the trace amounts of various builders listed in Table IV or by sodium silicates, which distributed into the sulfonate phase.

Table IV. Contamination of Isolated Sodium Sulfonate by Various Added Inorganic Salt Builders

(The inorganic salts were added to 9.0-gram portions of an unbuilt sodium C_{12}–toluene sulfonate detergent (35% sodium sulfonate) and the mixture was analyzed)

Inorganic Salt Builder		Inorganic Salt Builder Found in Deoiled Desalted Sulfonate Soln., g.
Formula	Amount added, g.	
$Na_6P_4O_{13}$	1.58	0.0032
Na_2HPO_4	2.25	0.0081
$Na_4P_2O_7$	2.25	0.0080
$Na_5P_3O_{10}$	2.25	0.0041
$Na_2B_4O_7$	0.84	0.0009
$NaBO_3$	0.57	0.0013

INORGANIC SALT. The applicability of the method to the determination of the inorganic salt content of a built detergent depends upon total solids content, which involves heating at 120° C. for 8 hours. The builder need not necessarily be soluble in the water–isopropyl alcohol mixture used for dissolving the sample, since such samples can be analyzed by deoiling a separate portion and obtaining a total solids value on the entire raffinate, containing undissolved builder.

Sodium bicarbonate decomposes on being heated; consequently, this salt should be converted to sodium sulfate, prior to a determination of total solids, by titration to a permanent phenolphthalein end point in boiling aqueous-alcoholic solution. Sodium perborate also decomposes on being heated (to give sodium borate); consequently, a separate determination of perborate in the original sample and in the total solids residue should be made and a correction applied for the decomposition of the perborate. The phosphates and the silicates are stable.

BASICITY OR ACIDITY. The inorganic salt builders under consideration are all basic to phenolphthalein, in aqueous or alcohol solution. Titration of these materials to the phenolphthalein end point would give an erroneous apparent sodium hydroxide content and the use of such a value would cause an error in the subsequent calculation of the inorganic salt content. Consequently, if a basic inorganic salt is present, it is preferable to omit the initial neutralization of the sample.

WATER. Bryant and his co-workers (*7*) found that water combined with several inorganic phosphates could be determined accurately by the Fischer method and it does not appear likely that any of the inorganic phosphate builders used in commercial detergents would interfere. However, sodium tetraborate and sodium perborate interfere in the direct determination of water by the Fischer method (*21, 22*). The correction factors for these salts are 7 and 3 moles of water per mole of salt, respectively.

APPLICATION TO COMMERCIAL DETERGENTS. Results obtained with several typical commercial sodium alkyl aryl sulfonate detergents which contain inorganic salt builders are given in Table V. The inorganic constituents were determined by conventional analytical methods. The values obtained for combining weight of the sodium sulfonate were those expected from products prepared from commercial C_{12}–C_{14} benzene alkylates. These materials were found free of alkyl sulfates by the application of the acid hydrolysis method (*10*).

Table V. Results Obtained in Analysis of Commercial Sodium Alkyl Aryl Sulfonate Detergents Containing Inorganic Salt Builders

Determination	A	B	C	D
Neutral oil, wt. %	1.0	0.5, 0.9	1.5	1.2
Sodium sulfonate, wt. %	18.0	29.4, 29.8	32.8	29.8
Inorganic salts, wt. %	75.7	66.4, 66.5	55.5	65.4
Sodium carboxylate, wt. %	0.6	.. ..	0.2	..
Acidity (as H_2SO_4), wt. %	..	0.2, 0.2	..	1.0
Water, wt. %	5.8	2.5, 2.6	11.4	2.3
Combining wt. of sodium sulfonate, grams per equiv.	355	355, 356	352	351
Inorganic constituents, wt. %				
Phosphorus, calcd. as $Na_5P_3O_{10}$	46.5	5.4	34.8	5.4
Silica, calcd. as $Na_2Si_3O_7$	5.0	..	4.5	..
Sulfate, calcd. as Na_2SO_4	14.5	58.9	14.7, 14.9	64.5

Analysis of Detergents Containing Organic Additives. SODIUM CARBOXYMETHYLCELLULOSE. In the analysis of commercial sulfonate detergents, any sodium carboxymethylcellulose present remains as a suspension during the neutralization and extraction procedures. During separation of the sodium sulfonate from inorganic salt, the sodium carboxymethylcellulose appears as an interfacial cuff. Since the amount present is usually small (1 to 2%), it does not interfere in the measurement of the volume of the alcohol phase or in the determination of sulfonate content or combining weight. To test the analytical behavior of this material, samples were prepared having an exaggeratedly high content (10%) of technical sodium carboxymethylcellulose and the mixture was analyzed by the method. Because of the large amount of suspended material present, the deoiled solution was centrifuged to remove the bulk of the sodium carboxymethylcellulose. As is shown in Table VI, close agreement was found between the determined and known values for neutral oil, sodium sulfonate content, and combining weight.

Table VI. Results Obtained in the Analysis of a Mixture of Sodium Carboxymethylcellulose and a Sulfonate Detergent

Determination	Content, Wt. %	
	Known	Found
Sodium carboxymethylcellulose	11.3[a] 10.0[a]	Not determined
Neutral oil	1.4 1.4	1.5 1.7
Sodium sulfonate	32.7 33.2	32.2 32.7
Combining wt. of sodium sulfonate, grams per equiv.	340 340	342 344

[a] As technical material.

NONIONIC DETERGENTS. In an attempt to apply the method to a sodium alkyl aryl sulfonate detergent containing an added nonionic detergent, Renex (a commercial nonionic detergent reported to consist of a mixture of polyethylene glycol esters of fatty and rosin acids), it was found that the nonionic detergent accompanied the sodium alkyl aryl sulfonate in the procedures; the two were found together in the deoiled desalted alcohol solution which normally contains only the sulfonate. The mixture of the two materials was separated by passing it, in aqueous-alcohol solution, through a cation exchange resin (Dowex 50 in the acid form) and an anion exchange resin (Amberlite IR-4B) in turn. The nonionic detergent isolated by this procedure contained less than 1% of sulfonate as determined by the methylene blue–cetyl pyridinium bromide method (*8*). The recovery of nonionic detergent was nearly complete. It was found possible to desorb the sulfonate adsorbed on the anion exchange resin by use of a 2% solution of sodium hydroxide in a 1 to 1 isopropyl alcohol–water mixture; a 96% recovery of sulfonate was obtained.

Analysis of Sodium Alkanesulfonate Detergents. Several members of this class of surface active material have been examined, the analytical data obtained being given in Table VII. These compounds had been prepared by means of oxidation of the corresponding mercaptans to the sulfonic acids. The alkanesulfonates behaved normally in the method; close agreement was found between the determined and theoretical combining weights. The quantity of sodium sulfonate found in the separated aqueous phases was somewhat less than with the sodium alkyl aryl sulfonates.

Table VII. Results Obtained in the Analysis of Sodium Alkanesulfates

Determination	Sodium 1-Decylsulfonate	Sodium 1-Dodecylsulfonate
Neutral oil, wt. %	1.0	0.5
Sodium sulfonate, wt. %	98.3	99.0
Inorganic salts, wt. %	0.5	0.4
Acidity (as H_2SO_4), wt. %	0.01	0.01
Water, wt. %	0.9	0.8
Total, wt. %	100.7	100.7
Combining wt. of sodium sulfonate, grams per equiv.		
Theor.	244	272
Det.	246	274
Organic material in separated aq. phase, calcd. as sodium sulfonate, as wt. % of orig. sample[a]	0.18	0.27
Na_2CO_3 content of desalted alc. phase, expressed as wt. % of total solid in alc. phase	0.62	0.49

[a] Calculated from carbon content of aqueous phase, determined by wet oxidation after removal of alcohol and carbonate.

Analysis of Sodium Alkyl Sulfate Detergents. The method has been found applicable to the analysis of sodium alkyl sulfate detergents, provided certain precautions are taken to ensure the stability of the alkyl sulfate during the drying periods. It is not possible to dry the sodium alkyl sulfate residues at 120° C., as they decompose at this temperature, turning brown and becoming acidic. The decomposition has been prevented by drying at a lower temperature and by maintaining basic conditions during the heating period, since the decomposition of alkyl sulfates is acid-catalyzed.

Several alkyl sulfate detergents, ranging from C_{10} to C_{18}, were analyzed employing these modifications. The analytical data obtained are given in Table VIII. Apparently satisfactory values were obtained in all determinations. Total values were generally close to 100%. The combining weight values were in close agreement with the theoretical values, indicating the stability of the sodium alkyl sulfates during the drying period. Combining weight was determined on the alkyl sulfate fractions both by the conventional ashing procedure and by acid hydrolysis (*10*). In this latter procedure the sulfuric acid liberated by hydrolysis is measured by titration with base and is a measure of the sulfate ester originally present.

In the neutral oil determination, only some 0.1% of sodium alkyl sulfates was removed by the petroleum ether extraction. This is somewhat less than that found with the sodium alkyl

Table VIII. Results Obtained in the Analysis of Sodium Alkyl Sulfate Detergents

Determination	Sodium-*n*-Decyl-1-sulfate	Sodium Cetyl Sulfate	Sodium Stearyl Sulfate	Sodium-*n*-Dodecyl-2-Sulfate	Sodium-*n*-Tetradecyl-2-sulfate	Sodium-*n*-Octadecyl-2-sulfate	Commercial Sodium Alkyl Sulfate Detergent
Neutral oil, wt. %	0.1	1.0	1.7	1.0	1.1	2.7	2.6, 2.6
Sodium alkyl sulfate, wt %	98.9	98.6	99.3	98.2	99.2	98.4	37.3, 38.0
Inorganic salts, wt. %	1.1	1.3	<0.1	2.3	0.7	<0.5	57.2, 57.5
Free base as NaOH, wt. %	...	...	...	0.01	...	0.01	.. ..
Free acid as H_2SO_4, wt. %	<0.01	<0.01	<0.01	...	<0.01	...	0.02, 0.02
Water, wt. %	0.5	0.1	0.2	0.09	0.07	0.1	...
Total, wt. %	100.6	101.0	101.2	101.6	101.1	101.2	..
Combining wt. of sodium alkyl sulfate, gram per equiv.							
Theoretical	260	344	373	288	316	373	...
Detd. by ashing	260	344	369	286	314	371	304, 318
Detd. by acid hydrolysis	267	251	371	288	314	373	...
Organic material in separated aq. phase, calcd. as wt. sodium alkyl sulfate, wt. % of orig. sample	0.6	...	...	0.07	0.09	0.26	...
Na_2CO_3 content of desalted alc. phase, wt. % of total solid in alc. phase	0.69	0.34	0.57	0.52	0.39	0.47	...

aryl sulfonate detergents. After separation of inorganic salts from the sodium alkyl sulfates, the resultant aqueous phase was found to contain only a small amount of sodium alkyl sulfate in the cases examined.

ACKNOWLEDGMENT

The authors wish to express their appreciation to S. A. Ballard, R. T. Holm, and D. S. Melstrom of the Shell Development Co. for many of the surface active materials investigated.

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RECEIVED for review June 19, 1954. Accepted October 4, 1954.

Titration of Phenolic Esters in Ethylenediamine

R. A. GLENN[1] and JANET T. PEAKE[2]

Coal Research Laboratory, Carnegie Institute of Technology, Pittsburgh, Pa.

The titration of weak acids in nonaqueous media has become an active field of investigation in recent years and is being widely applied in the determination of phenols in resins, tars, coal hydrogenation products, and similar materials. In the present work the scope of acid-base titrations in ethylenediamine has been extended to include phenolic esters.

IN RECENT years improved procedures and techniques have been reported for making both potentiometric and visual titrations of a variety of weak acids in nonaqueous media. Sodium aminoethoxide in ethylenediamine (*8*, *9*) and sodium methoxide in benzene-methanol (*6*), all have been reported as being satisfactory titrants. Azo violet has been employed as the indicator for the visual titration of salts (*3*) and sulfonamides (*4*) in ethylenediamine, in dimethylformamide, and in butylamine, as well as negatively substituted phenols in dimethylformamide. Also, *o*-nitroaniline has been reported as a satisfactory indicator for the titration of phenols and alkyl-substituted phenols in ethylenediamine (*5*).

In this paper the scope of acid-base titrations in ethylenediamine is extended to include the determination of phenolic esters. Both the colorimetric procedure of Fritz and Keen using potassium methoxide in benzene-methanol with *o*-nitroaniline or azo violet as indicator (*5*) and the automatically recorded potentiometric procedure of Katz and Glenn (*8*) using sodium aminoethoxide as titrant have been used to titrate a number of phenolic esters alone and in mixtures with ethylenediamine as the solvent.

REAGENTS AND SOLUTIONS

In general, the solvents and the reagents as described by Katz and Glenn (*8*) or by Fritz and Keen (*5*) were used.

Benzene, ACS grade.

Methanol, ACS grade.

Potassium acid phthalate, primary standard grade.

Benzoic acid, primary standard grade.

Ethylenediamine, 98 to 100%, Matheson Coleman and Bell, or anhydrous, used as received.

Ethanolamine, Eastman White Label, redistilled before use.

o-Nitroaniline, Eastman, 1.5 grams dissolved in 100 ml. of benzene.

Azo violet (*p*-nitrobenzeneazoresorcinol), Matheson Coleman and Bell, saturated solution in benzene.

Phenol samples, all commercial samples except as indicated.

Potassium methoxide, 0.1*N*. About 4 grams of potassium were dissolved in 20 ml. of methanol and 50 ml. of benzene in a loosely stoppered flask. Methanol was added until the solution became homogeneous; next benzene was added until the solution became cloudy; then more methanol, until the solution cleared again. This dilution procedure was repeated until 1 liter of clear solution was obtained (*5*). It was standardized against benzoic acid.

Sodium aminoethoxide, 0.2*N*. About 0.4 gram of sodium was dissolved in 20 ml. of ethanolamine, diluted to 100 ml. with ethylenediamine (*9*), and standardized against salicylic acid, benzoic acid (*8*), and potassium acid phthalate.

PROCEDURES

Procedure A. Approximately 2 drops of *o*-nitroaniline indicator were added to 20 ml. of ethylenediamine and titrated to an orange-red color with potassium methoxide, after which about 0.5 meq. of sample was added quickly, and the titration was continued to the reappearance of the orange-red color (*5*).

Procedure B. Approximately 0.1 meq. of sample was dissolved in 10 ml. of ethylenediamine and titrated potentiometrically using the continuous automatic titration assembly of Katz and Glenn equipped with glass-platinum electrodes (*8*).

Procedure C. Approximately 2 drops of azo violet indicator were added to 20 ml. of ethylenediamine and titrated to a clear blue color with potassium methoxide. Then approximately 0.5 meq. of sample was introduced quickly and the titration was continued to the reappearance of the blue color (*5*).

SCOPE

Phenolic esters of carboxylic acids undergo aminolysis, in excess ethylenediamine at room temperature, producing phenols and amides of ethylenediamine. Lactones of phenols also undergo aminolysis. The generalized equations may be written:

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[1] Present address, Mellon Institute, Pittsburgh, Pa.

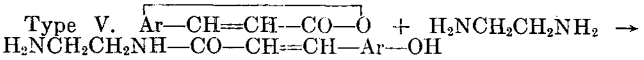
[2] Present address, Mount Holyoke College, South Hadley, Mass.

Type I. $Ar—O—CO—R + H_2NCH_2CH_2NH_2 \rightarrow Ar—OH + H_2NCH_2CH_2NH—CO—R$

Type II. $Ar—O—CO—Ar' + H_2NCH_2CH_2NH_2 \rightarrow Ar—OH + H_2NCH_2CH_2NH—CO—Ar'$

Type III. $Ar—O—CO—Ar'—OH + H_2NCH_2CH_2NH_2 \rightarrow Ar—OH + H_2NCH_2CH_2NH—CO—Ar'—OH$

Type IV. $HOOC—Ar—O—CO—R + H_2NCH_2CH_2NH_2 \rightarrow HOOC—Ar—OH + H_2NCH_2CH_2NH—CO—R$

Type V.

$Ar—CH{=}CH—CO—O + H_2NCH_2CH_2NH_2 \rightarrow H_2NCH_2CH_2NH—CO—CH{=}CH—Ar—OH$

The carboxylic acid may be either aliphatic or aromatic in character and the phenol either benzenoid or naphthalenic. The titration of the reaction mixture by the procedures described above determines only the phenolic groups present either in the original ester molecule or in the aminolysis products.

The effects of structure on the aminolysis of esters have been investigated by Day and coworkers. Of the various amines studied, ethylenediamine was found to be second only to methylamine in reactivity (*1*) and of the various types of esters studied, phenolic esters were found to be the most reactive (*7*).

In a study of the aminolysis of γ-lactones, Bistrzycki and Schmutz (*2*) found that thel actone of $o—HO—C_6H_4CH_2COOH$, commonly called phthalide, and the lactone of $o—HO—C_6H_4—CH(C_6H_5)COOH$ readily underwent aminolysis with ethylenediamine. Furthermore, the rate of reaction of the latter lactone with even 50% aqueous ethylenediamine was so rapid that alcohol had to be added to keep the reaction under control.

Although alcoholic esters may undergo aminolysis with ethylenediamine at room temperature, they do not yield acidic products and consequently are not titratable by the procedures outlined above.

The feasibility of determining phenolic esters by a simple direct titration in ethylenediamine has been demonstrated by the analysis of 15 esters representing five different types as listed in the generalized equations. Either or both of two different titration techniques which have been described previously in the literature were used: one, a rapid visual indicator procedure (*5*) for use with colorless materials, and the other, a continuous, automatically recorded potentiometric procedure (*8*) for use with colored materials and with mixtures, or wherever an automatically recorded titration curve is desirable. Most of the data reported in this paper were obtained with the latter procedure. In the discussion of these results below, the phrase "titration of esters" is used for the sake of brevity to mean the titration of acidic groups originally present in the ester or formed by aminolysis rather than the titration of the ester group itself.

VISUAL INDICATOR PROCEDURE

o-Nitroaniline, which has been used by other workers (*5*) as an indicator in the titration of phenols, was successfully used in this work to determine esters of both Types 1 and 2 by Procedure A (see Table I). Reproducible results were obtained without difficulty in the titration of eight phenolic esters of both aliphatic and aromatic carboxylic acids. However, reproducible results could not be obtained in the titration of diphenyl succinate because of fading of the indicator. Both diphenyl phthalate and coumarin produced colored solutions upon being dissolved in ethylenediamine and thus could not be titrated visually; however, they were titrated potentiometrically by Procedure B as described below. Difficulties were experienced also in the titration of phenyl chloroacetate, apparently as a result of dehydrohalogenation which is also discussed more fully below.

Azo violet can be used as an indicator in the determination of carboxylic acids and phenols with negative substituents in the ortho and para positions (Procedure C) (*5*), but not in the determination of weak phenols. Procedure C was used in conjunction with Procedure A to determine differentially the titer of each of the two phenolic aminolysis products of phenyl salicylate (see Table I). Procedure A was used to determine the total titer and Procedure C to determine the titer of only the hydroxyl group of the ethylenediamine amide of salicylic acid. The titer for phenol produced by the aminolysis was then obtained by difference.

Table I. Colorimetric Titration of Phenolic Esters by Procedure A

Compound	Type	Amount Taken, μ mole	Amount Found		
			μeq.	%, Theory	Average, %, theory
Phenyl acetate	I	(1) 366.0	360.8	98.6	99.0
		(2) 303.5	301.5	99.3	
o-Tolyl acetate	I	(1) 339.1	341.3	100.6	
		(2) 397.9	401.9	101.0	101.0
		(3) 356.1	361.2	101.4	
m-Tolyl acetate	I	(1) 398.7	400.1	100.4	.100.5
		(2) 333.7	335.3	100.5	
α-Naphthyl acetate	I	(1) 326.6	328.3	100.5	
		(2) 364.9	366.4	100.4	100.3
		(3) 349.2	349.5	100.1	
Phenyl propionate	I	(1) 301.9	309.2	102.4	
		(2) 340.1	340.8	100.2	100.1
		(3) 365.2	364.2	99.7	
Phenyl benzoate	II	(1) 419.2	419.3	100.0	
		(2) 373.5	379.1	101.5	100.6
		(3) 381.5	382.2	100.2	
o-Tolyl benzoate	II	(1) 392.1	392.4	100.1	
		(2) 374.6	374.6	100.0	100.1
p-Tolyl benzoate	II	(1) 355.1	361.4	101.8	
		(2) 295.6	297.8	100.7	101.0
		(3) 342.7	344.5	100.5	
		Procedures A and C			
			Procedure A, Total Titer		
Phenyl salicylate	III	(1) 147.9	293.7	198.6	
		(2) 143.2	290.8	203.1	201.7
		(3) 161.7	330.0	204.0	
		(4) 192.7	387.4	201.0	
			Procedure C, Strong Phenol Titer		
		(1) 349.1	405.4	116.1	116.0
		(2) 347.9	403.3	115.9	
		Total titer		201.8%	
		Strong phenol titer		116.0%	
		Phenol produced by aminolysis		85.8%	

Ethylenediamine, being on hand and immediately available, was used by a solvent for Procedure C instead of the dimethylformamide recommended by Fritz and Keen (*5*) for the titration of strong simple phenols using azo violet as indicator. Check titrations of benzoic acid using the potentiometric system and the indicator simultaneously showed that the indicator changed at the point of maximum change in e.m.f.

POTENTIOMETRIC PROCEDURE

The precision of the potentiometric procedure (Procedure B) is not as high as that of the visual indicator method (Procedure A) for two reasons. First, the automatically recorded titration curve obtained by Procedure A can be read to only the nearest chart division. Under the conditions normally used, an error in reading the chart by one chart division corresponds to an error in the determination of about 1.5%. Second, the correction for a separately determined blank on the solvent introduces an error which is avoided in Procedure A by the titration of the solvent prior to introduction of the sample. Nevertheless, the precision of Procedure B, being approximately 1.5%, is satisfactory for the method to be used in many fields of analysis, particularly in the analysis of colored materials and of mixtures.

Eleven esters were titrated singly using Procedure B. Several of these were also titrated in mixtures with compounds of known relative acidic strength.

In the titration of acidic oxygen compounds in ethylenediamine it was found that acids generally fall into one of four distinct acidic strength groups which can be differentiated by Procedure B: (*a*) carboxylic acids comparable in strength to carbon dioxide, (*b*) weak carboxylic acids and negatively substituted phenols, (*c*) phenol and alkyl-substituted phenols, and (*d*) weak phenols which have a deactivating group on the ring. Because compounds

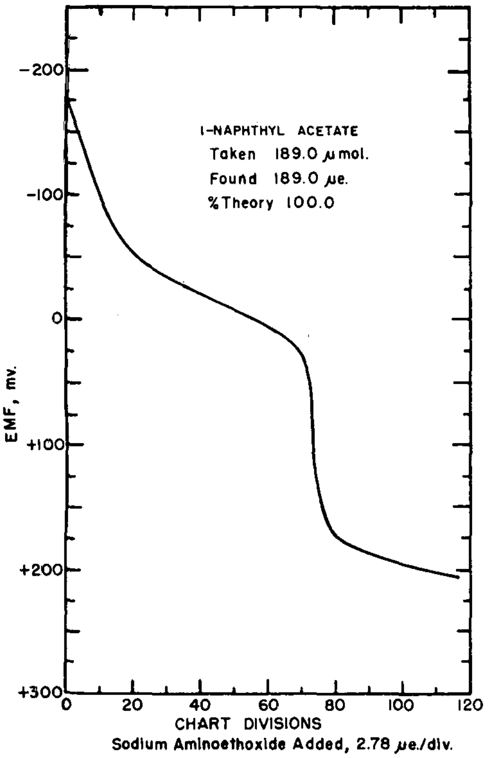


Figure 1. Potentiometric titration of 1-naphthyl acetate

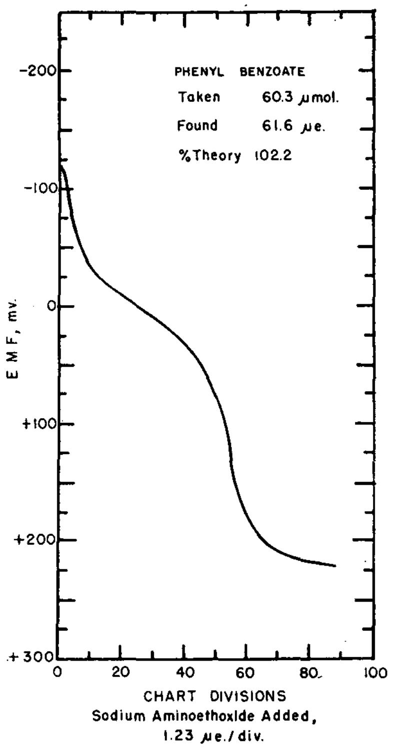


Figure 2. Potentiometric titration of phenyl benzoate

Table II. Potentiometric Titration of Individual Phenolic Esters by Procedure B

Compound	Type	Amount Taken, µ mole	Amount Found: Strong Acid, µeq.	Strong Acid, %, theory	Weak Acid, µeq.	Weak Acid, %, theory
Diphenyl succinate	I	63.7	...	...	125.5	98.5
		83.5	...	...	169.2	101.3
α-Naphthyl acetate	I	189.0	...	...	189.0	100.0
		70.7	...	...	71.9	101.7
Phenyl stearate	I	48.2	...	...	39.3	81.5
Phenylchloroacetate	I	180.0	176.0	97.8	173.5	96.4
Phenyl benzoate	II	55.2	...	...	57.8	104.7
		60.3	...	...	61.6	102.2
p-Tolyl benzoate	II	59.3	...	...	60.8	102.5
		51.7	...	...	52.3	101.2
Diphenyl phthalate	II	31.6	...	...	62.6	99.1
		33.1	...	...	65.6	99.1
Phenyl salicylate	III	43.7	45.6	104.3	45.6	104.3
		170.0	175.8	103.4	175.2	103.1
Acetyl salicylic acid	IV	70.0	74.4	106.3	74.4	106.3
		63.9	68.2	106.7	68.2	106.7
Coumarin	V	159.5	158.5	99.4	...	...
		175.5	175.1	99.8	...	...
6-Dibenzopyrone	V	52.3	51.3	98.1	...	...

of the same acidic strength will titrate together to give a single end point, simple acids of representative Groups *b*, *c*, and *d* were titrated with esters to check qualitatively the acidic strengths of the aminolysis products. The representative acids were potassium acid phthalate (Group *b*), benzoic acid (Group *b*), salicylamide (Group *b*), 3,5-xylenol (Group *c*), and salicylic acid (Group *b* and Group *d*). Carbon dioxide is the only known example of Group *a*, and as the amount of it present in the solvent is kept at a minimum, it is best determined by a separate blank titration on the solvent alone. No titrations were made with large amounts of carbon dioxide present.

When a compound of intermediate strength is titrated in a mixture which contains one or more of the representative compounds of close acidity, differentiation is not possible. For example, a two-component mixture of a compound of strength intermediate between Groups *b* and *c* and a compound of Group *b* will titrate to give a single end point. The same phenomenon will be observed if the components of the mixture are a Group *c* compound and the intermediate. If all three are titrated in a mixture, only one gradual e.m.f. break will be observed. Thus, by addition of suitable representative compounds, the relative strength of a given acid can be determined.

In the discussions of the titration curves obtained by Procedure B, reference to the first e.m.f. end point means the end point following the neutralization of the most acidic compounds introduced, and not the end point for the neutralization of the carbon dioxide originally present in the solvent.

Type I Esters. The aminolysis of esters of Type I (Ar—O—CO—R) yield a monobasic phenol, and consequently only one end point is observed in the titration of the products of this type of phenolic ester. Diphenyl succinate and α-naphthyl acetate, examples of this type of ester, are listed in Table II and were found to titrate without difficulty. The automatically recorded titration curve for α-naphthyl acetate is shown in Figure 1. If R is small, no difficulty in titration is experienced, but if R is large in size the ester becomes difficultly soluble in ethylenediamine. The solubility of phenyl stearate is visibly decreased and less than theoretical values are obtained when it is titrated (see Table II).

Phenylchloroacetate, however, exhibits two end points when titrated potentiometrically. In addition to the weak end point normally observed in the titration of simple phenols, an end point characteristic of a much stronger acid is observed first. The chlorine of the phenylchloroacetate becomes ionized upon solution of the ester in ethylenediamine and apparently forms a titratable acid which is much stronger than the phenol produced by aminolysis. The ionization of the chlorine was established

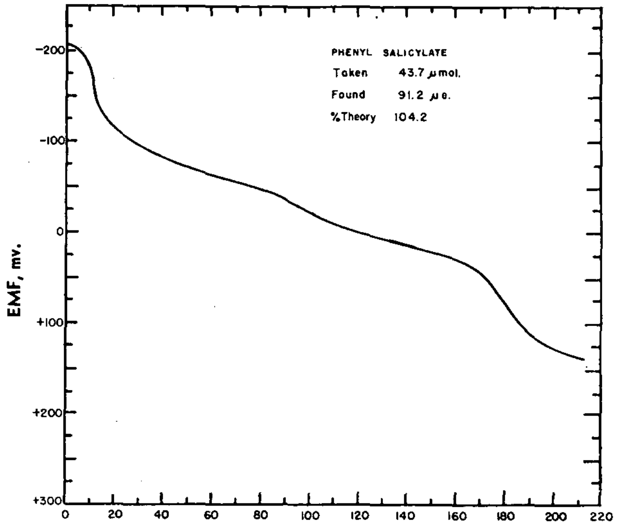


Figure 3. Potentiometric titration of phenyl salicylate

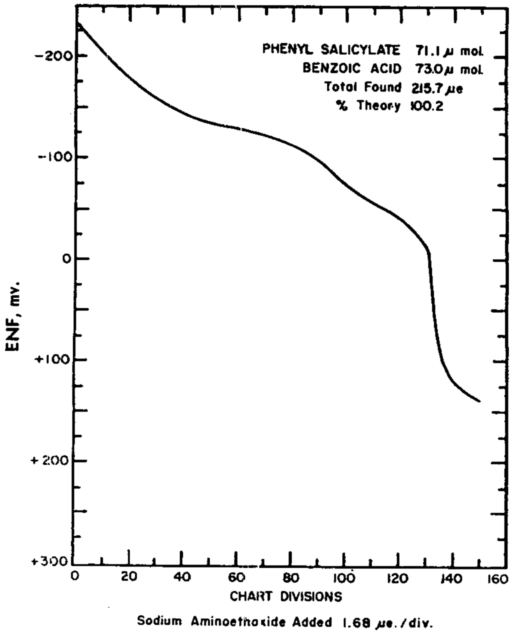


Figure 4. Potentiometric titration of a mixture of phenyl salicylate and benzoic acid

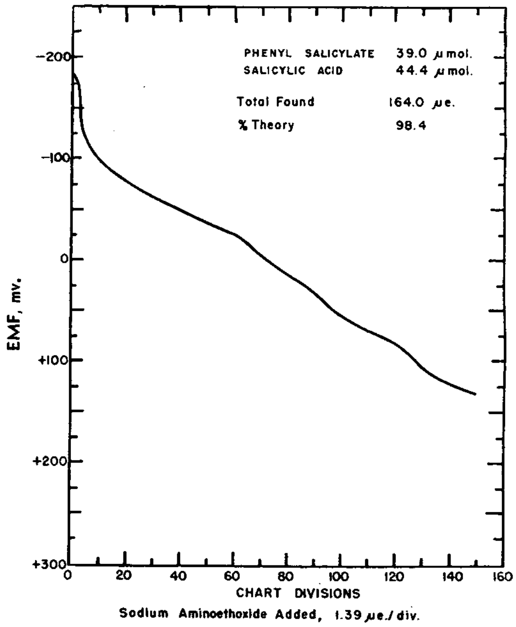


Figure 5. Potentiometric titration of a mixture of phenyl salicylate and salicylic acid

in a separate experiment by the rapid formation of a precipitate when silver nitrate solution was added to an ethylenediamine solution of phenylchloroacetate which had been diluted and then acidified with nitric acid.

Type II Esters. Monobasic phenols are also produced on aminolysis of Type II esters (Ar—O—CO—Ar′) and again only one end point is observed when this type of ester is titrated. Phenyl benzoate (Figure 2), *p*-tolyl benzoate, and diphenyl phthalate are examples of esters of this type which were titrated without difficulty (see Table II). The two molecules of phenol which are produced in the aminolysis of diphenyl phthalate were found to titrate simultaneously. A mixture of phenyl benzoate and 2,3,5-trimethylphenol produces only one sharp end point which corresponds to the theoretical titer for the sum of the two phenolic compounds (see Table III).

Type III Esters (Ar—O—CO—Ar′—OH). Phenyl salicylate was the only ester of Type III which was titrated. The aminolysis products of this ester would be phenol and an *N*-substituted salicylamide. Since phenols with negative substituents in the ortho position are comparable in strength to carboxylic acids (*5*), the phenolic group of the *N*-substituted salicylamide would be expected to titrate, in this case, as a strong acid. In accord with this, two end points were observed in the titration of this ester (Figure 3).

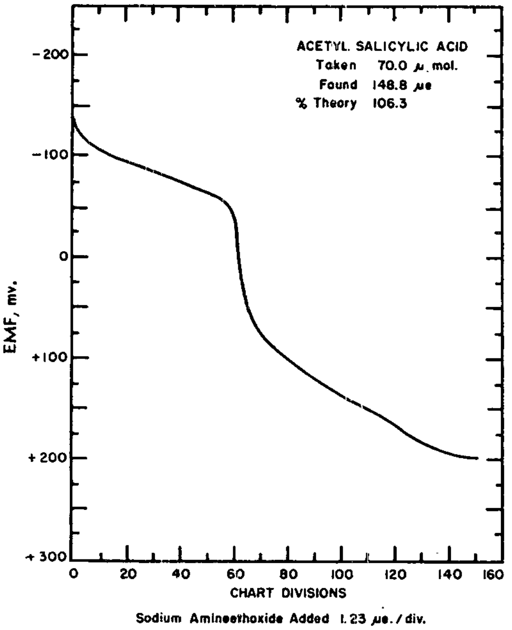


Figure 6. Potentiometric titration of acetyl salicylic acid

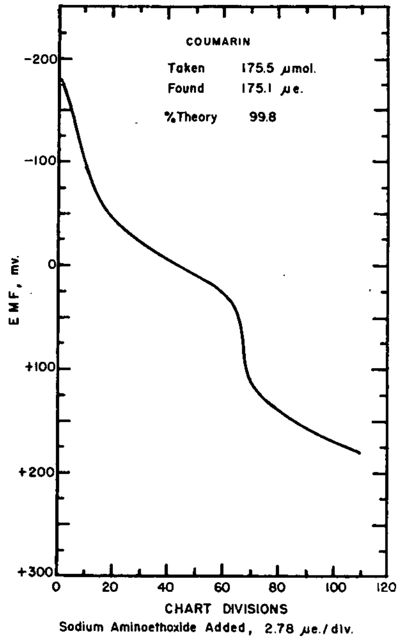


Figure 7. Potentiometric titration of coumarin

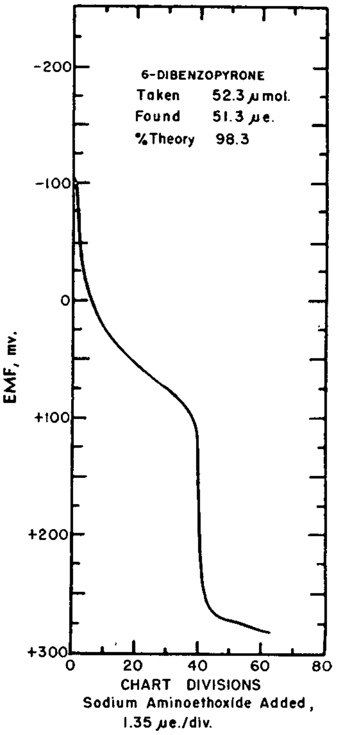


Figure 8. Potentiometric titration of 6-dibenzopyrone

Phenyl salicylate was also titrated in mixtures (Table III) with the following: (*a*) benzoic acid, (*b*) salicylamide, (*c*) 3,5-xylenol, (*d*) salicylamide and 3,5-xylenol, and (*e*) salicylic acid. Both benzoic acid (Figure 4) and salicylamide increased the titer prior to the first end point which is that for acids of Group *b*. 3,5-Xylenol, being an alkylated phenol and thus a Group *c* acid, increased the titer due to phenol from aminolysis of the ester—i.e., the titer between the first and second end points. Addition of both salicylamide and 3,5-xylenol to phenol salicylate increased the titer for both the strong and the weak acids, the strong being increased by the amount of salicylamide added, and the weak by the amount of xylenol added. The addition of salicylic acid to the phenyl salicylate increased the titer between the first and second end points and introduced a third end point, the difference between the second and third end points corresponding to the amount of salicylic acid added (Figure 5). The appearance of the third end point indicates that the hydroxyl group of the salicylic acid is considerably weaker than the phenol from aminolysis of the phenyl salicylate, and thus is classed as Group *d* in strength.

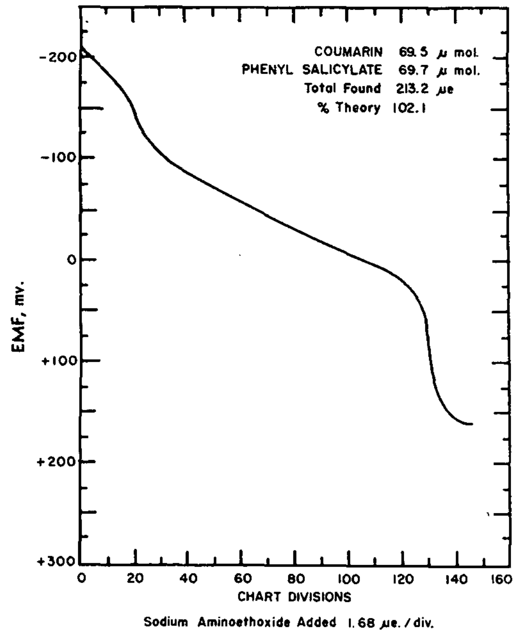


Figure 9. Potentiometric titration of a mixture of coumarin and phenyl salicylate

Type IV Esters (R—CO—O—Ar—COOH). Acetyl salicylic acid, the only ester of this type titrated, yields salicylic acid as the acidic product upon aminolysis, and, as expected, two end points are obtained (Figure 6).

Type V Esters (Lactones). Coumarin and 6-dibenzopyrone were titrated as examples of lactones of phenols and in each case only one end point was observed (Figures 7 and 8). In addition, coumarin was titrated in mixtures with (*a*) potassium acid phthalate, (*b*) benzoic acid, (*c*) salicylamide, (*d*) 3,5-xylenol, and (*e*) phenyl salicylate. In each case, including the latter (Figure 9), only one end point was observed indicating that the aminolysis product of coumarin has an acidity between that of carboxylic acids and phenols.

Miscellaneous Compounds. Phenolic ethers are not split by ethylenediamine at room temperature—anisole and diphenylene oxide do not titrate. Sulfonic acid esters of phenols also do not undergo aminolysis at room temperature—phenyl *p*-toluene sulfonate does not titrate.

Table III. Data on Potentiometric Titration of Mixtures Containing Phenolic Esters by Procedure B

			Amount Found							
			First End Point		Second End Point		Third End Point		Total	
Components	Type	Amount Taken, μmole	μeq.	%, theory	μeq.	%, theory	μeq.	%, theory	μeq.	%, theory
Phenyl benzoate 2,3,5-trimethyl pheno	II	25.3 39.9 65.2	66.3	101.7	...	...	...	...	66.3	101.7
Phenyl salicylate Benzoic acid	III	71.1 73.0 144.1	150.2	104.2	65.5	92.1	...	...	215.7	100.2
Phenyl salicylate Salicylamide	III	72.9 66.3 139.2	142.7	102.5	70.6	96.8	...	...	212.3	100.1
Phenyl salicylate 3,5-Xylenol	III	54.5 49.2 103.7	54.4	99.8	105.2	101.4	...	...	159.7	100.9
Phenyl salicylate Salicylamide 3,5-Xylenol	III	54.0 55.4 57.2 166.6	108.8	99.5	112.7	101.3	...	...	221.5	100.4
Phenyl salicylate Salicylic acid	III	40.0 41.7 81.7	81.5	99.8	37.6	94.0	43.6	104.6	161.7	99.0
Phenyl salicylate Salicylic acid	III	39.0 44.4 83.4	82.0	98.3	37.5	96.2	44.5	100.2	164.0	98.3
Coumarin Potassium acid phthalate	V	67.5 59.8 127.3	130.2	102.3	...	...	...	...	130.2	102.3
Coumarin Benzoic acid	V	87.0 71.6 158.6	157.2	99.1	...	...	...	...	157.2	99.1
Coumarin Salicylamide	V	138.2 142.5 280.7	279.0	99.4	...	...	...	...	279.0	99.4
Coumarin 3,5-Xylenol	V	142.8 155.3 298.1	295.0	99.0	...	...	...	...	295.0	99.0
Coumarin Phenyl salicylate	V III	69.5 69.7 139.2	213.2	102.1	...	...	...	...	213.2	102.1

ACKNOWLEDGMENT

The authors wish to thank the Carbide and Carbon Chemicals Co. of South Charleston, W. Va., for the supply of anhydrous ethylenediamine used in this work.

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RECEIVED for review July 31, 1954. Accepted November 13, 1954.

Coulometric Titration of Dyes with Externally Generated Titanous Ion

J. S. PARSONS and WILLIAM SEAMAN

Research Division, American Cyanamid Co., Bound Brook, N. J.

Titanous ion has been generated electrolytically at a mercury cathode in an external electrolysis cell using constant current. The average current efficiency of 31 titrations was 99.8%, with a standard deviation for a single value of ±0.15%. The generated reagent has been used to titrate Orange II, tartrazine, *p*-aminoazobenzene, amaranth, and methyl violet with results in satisfactory agreement with the conventional volumetric titanous chloride titration methods.

TITANOUS chloride is used extensively for titration of dyes. Coulometric titration with electrolytically generated titanous ion has a particular advantage, as titanous chloride reagent requires frequent standardization because of its instability. Arthur and Donahue (*1*) have reported the internal generation of titanous ion at a gold electrode. However, the necessity for frequent reconditioning of the electrode so as to maintain a high current efficiency proved too bothersome in the authors' hands.

Titanous ion generation with 100% efficiency is difficult to achieve because of the competing reaction, $H^+ + e = {}^1/_2\, H_2$, the redox potential of which is close to that of the titanous-titanic system. In this work external generation of titanous ion at a mercury pool cathode was used. The use of externally generated reagents was first described by De Ford, Pitts, and Johns (*3*). Mercury, which has a much higher hydrogen overvoltage than gold, was found to be a better electrode material. The external electrolysis cell was preferable to internal generation, since the near-boiling temperature at which some titrations of dyes are carried out caused certain difficulties in using internal generations at the mercury cathode. Furthermore, external generation allows conditions for 100% efficient electrolysis to be standardized so that in setting up the coulometric method any necessary changes in the present dye titration procedures can be minimized.

APPARATUS AND REAGENTS

Instrumentation. Precision coulometric titration equipment which was constructed in this laboratory will be described in another paper. The essential components consist of a Reilley, Adams, and Furman constant current supply (*7*), which was modified so that currents up to 450 ma. could be obtained and a clock (Standard Electric Time Co. Model S-1 with direct current clutch) which is operated by a Riverbank standard tuning fork (Cenco, 60 cycles, 5 p.p.m. precision). The current was measured from the potential drop across a standard resistor in the circuit by means of a Rubicon portable precision potentiometer (Catalog No. 2710). Other equipment (*3*) would be satisfactory for the current levels used in this work. It was, however, necessary to use a frequency standard to operate the timer in the work reported here, because of frequency variations in the plant power.

Electrolysis Cell. A diagram of the electrolysis cell used is shown in Figure 1. Approximately 0.4*M* titanium tetrachloride solution is allowed to pass in a thin layer over a mercury pool cathode which is separated from the anode compartment by a fritted-glass disk. The titanous ion produced at the cathode is carried to the titration beaker by a capillary tube. The flow rate is adjusted by keeping the electrolysis solution under slight nitrogen pressure with a mercury pressure regulator.

A stopcock shell made a convenient cell (*6*). Rubber stoppers were used to connect the tube containing the fritted-glass disk and the mercury pool assembly to the stopcock as illustrated in Figure 1. The capillary tubes were fitted inside the stopcock tubes, so that the capillaries were flush with the inner wall of the stopcock shell. The mercury pool, which has an area of about 1 sq. cm., was adjusted so that the distance between the pool and fritted glass tube was about 1 mm. or less (mercury should not touch the fritted-glass disk). A tube with a coarse porosity fritted disk was employed. Seepage was made very slow (less than 1 ml. in several hours) by allowing some of the titanic solution to hydrolyze so as to clog the pores. The fritted-glass disk becomes clogged when titanium ion is generated for about 20 minutes at 260 ma. with the titanic solution flowing through the cell at a slow flow rate (about 2 ml. per minute). A fritted-glass tube prepared in this way should be satisfactory for many weeks, provided the tube is not allowed to dry out. The tube may be unclogged by boiling in concentrated sulfuric acid. A zinc rod was used as the anode with 5% sodium sulfate as the anolyte. The zinc rod should be fitted tight at the upper end, so that the titanium tetrachloride solution cannot be forced into the anode compartment. The anolyte solution was renewed for each titration. It is recommended that for best results a fresh mercury pool be used for each analysis. The mercury pool can be removed by releasing the rubber stopper at the bottom of the cell, then replacing the stopper and allowing fresh mercury to flow from the reservoir. Titanium tetrachloride solution should not be allowed to stand in the cell overnight, as it will hydrolyze. It is recommended that the electrolysis cell assembly be installed on the movable stand (*8*) (available from Scientific Glass Co., Bloomfield, N. J.), so that the delivery tube can be raised or lowered into the titration vessel with ease.

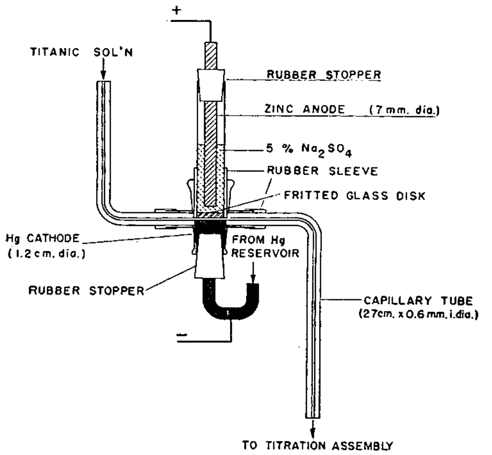

Figure 1. Electrolysis cell

Reagents. The stock titanic chloride solution was prepared according to the procedure described by Arthur and Donahue (*1*). Water was added slowly to 200 ml. of C.P. titanic chloride (Amend Drug & Chemical Co., New York) with stirring until vigorous hydrolysis ceased. The mixture goes through a doughy stage, making stirring difficult. However, by macerating with a large stirring rod and adding more water, complete solution is obtained. The final solution was diluted to 500 ml. This solution is approximately 3.6*M* in titanic ion and is stable to hydrolysis for at least 6 months. A small amount of titanium dioxide formed by hydrolysis may settle out, but this is of no consequence, as the supernatant liquor is clear. The stock titanium tetrachloride solution gave a negative test for ferric ion when 2.5 ml. of stock solution was diluted to 50 ml. in a Nessler tube and treated with 2.5 grams ammonium thiocyanate. The pale yellow color of this solution was definitely distinguishable from the red tint of a similar solution to which had been added 0.0007 mg. of ferric ion.

The electrolysis solution was prepared by diluting the concentrated stock ten times with distilled water. This solution, which had a pH of 0.6, began to hydrolyze seriously in 12 to 24 hours. Hence, the solution must be used within an hour or two after preparation. Nitrogen (prepurified grade) should be bubbled through the solution for a few minutes before use in order to remove dissolved oxygen.

The standard potassium dichromate solution used for testing the efficiency of titanous ion generation was prepared by weighing out National Bureau of Standards potassium dichromate and making up to volume in carefully calibrated glassware. Based upon the weight of potassium dichromate this solution was 0.02504*N;* by coulometric titration with electrolytically generated ferrous ion (*2*) five titrations gave 0.02500, 0.02502, 0.02509, 0.02508, 0.02501; average, 0.02504.

A triple-distilled grade of mercury was used.

METHOD FOR TESTING EFFICIENCY OF TITANOUS ION GENERATION

A 200-ml. tall-form electrolysis beaker was provided with a magnetic stirrer and rubber stopper, so that an atmosphere of carbon dioxide could be maintained. Approximately 35 ml. of 6*N* sulfuric acid were added and carbon dioxide (purified by passage through a chromous solution) was bubbled through the solution for a few minutes. Then after 3 grams of reagent grade Mohr's salt crystals had been added and dissolved, a 25-ml. aliquot (calibrated pipet) of 0.02504*N* potassium dichromate was added. Carbon dioxide was bubbled through the solution for a few minutes and finally carbon dioxide was allowed to pass over the surface of the solution during the titration.

The electrolysis delivery tube was lowered into the titration beaker so that the tip was immersed in the solution. The flow of titanic chloride reagent (8 to 9 ml. per minute) was started and the electrolysis current was switched on to begin the titration. Near the end point 10 ml. of 6.6*M* ammonium thiocyanate indicator were added and the current was then interrupted at 0.25-second intervals until the reddish ferric thiocyanate color was just discharged. A blank was run in exactly the same way, substituting 25 ml. of distilled water for the dichromate solution and adding about the same volume of titanic chloride solution as for the titration. The efficiency was calculated as follows:

$$\frac{\text{Milliequivalents of potassium dichromate taken} \times 96.5 \times 100}{\text{Time for titration in seconds} \times \text{current in amperes}} = \%\ \text{efficiency}$$

PROCEDURE FOR DYE TITRATIONS

Weigh 5 grams of sodium bitartrate ($NaHC_4H_4O_6 + H_2O$) into the titration beaker and add 50 ml. of distilled water. Start the flow of carbon dioxide through the beaker and heat to boiling for a few minutes to remove dissolved oxygen. Lower the electrolysis cell delivery tube into the beaker with the flow of titanium tetrachloride solution adjusted to 8 to 9 ml. per minute. Switch on the current (adjusted to 260 ma.) and generate titanous ion for a few seconds so as to reduce any reducible substances in the reagents. Remove the electrolysis assembly and boil for 3 minutes. Back-titrate the excess titanous ion with 0.025*N* amaranth to a faint pink which persists for 30 seconds. To avoid the back-titration, the standard amaranth solution may be added with a Mohr pipet to give a permanent red, indicating the presence of excess amaranth; then the solution may be brought to an exact end point by generating titanous ion just to decolorize the amaranth.

Pipet into the beaker a 25-ml. aliquot of the dye solution—e.g., 2.000 grams of Orange II dissolved and made up to 1 liter in distilled water. Bring to a boil with carbon dioxide bubbling through the solution. Commence the titration, keeping an atmosphere of purified carbon dioxide above the titration solution at all times and the temperature just under the boiling point. The end point is taken, for instance, in the case of Orange II, when the orange color is just discharged or when a 0.25-second addition of titanous ion causes no further change in color in the solution. The titration required about 190 seconds.

Other dyes require variations in this procedure. With aminoazobenzene, tartrazine, and methyl violet excess titanous ion is generated and the excess is back-titrated to a pink tinge with 0.025*N* amaranth. The amaranth may be added with a pipet and titanous ion generated just to decolorize the excess amaranth, as previously described. The aminoazobenzene hydrochloride is made up in 3A alcohol and the titration is carried out in approximately 35% 3A alcohol–water medium. In the case of tartrazine and methyl violet, 15 grams of sodium bitartrate are used.

RESULTS AND DISCUSSION

Values of the current efficiencies for external generation of titanous ion in the cell shown in Figure 1 at several current levels are presented in Table I. The average current efficiency and the standard deviation of a single value calculated from all the data in Table I were 99.8 and ±0.15%.

Factors which tend to affect the current efficiency are:

Table I. Current Efficiency for External Generation of Titanous Ion at Mercury Cathode

Current, Ma.	Flow Rate, Ml./Min.	No. of Detns.	Efficiency, %	Standard Deviation
111.0	8-9	4	99.95	0.2
158.1	8-9	2	99.94, 100.02	..
260.4	8-9	17	99.76	0.14
297.0	8-9	2	99.97, 99.92	..
297.0	15	4	99.94	0.16
420.0	15	2	99.59, 99.89	..

The hydrogen ion concentration of the titanium tetrachloride electrolysis solution should not be increased. The approximately 0.4*N* titanium tetrachloride solution as made up has a pH of 0.6. A titanium tetrachloride solution which was stabilized by adjusting the acidity to approximately 1*N* in sulfuric acid gave a low current efficiency. A solution of 0.4*N* titanic sulfate (pH = 0.4) gave a rapid evolution of hydrogen at the mercury pool.

A fast flow rate through the cell is desirable in order to keep the depolarizer concentration at the electrode surface high. Hydrogen was evolved at the mercury pool when the flow rate was decreased to less than 2 to 3 ml. per minute.

The hold-up volume in the electrolysis cell and delivery tube must be kept as small as possible for better flow and flushing characteristics.

A bright mercury surface is important for best results. Repeated use of the mercury pool without renewing the surface may lead to low efficiencies. The titanium tetrachloride solution should not be allowed to hydrolyze in the cell. Caution should be taken to ensure that traces of metals or other impurities do not get into the titanium tetrachloride reagent.

Internal Versus External Generation. One advantage of internal electrolysis is the large electrode surface that can be used, so that the current density can be kept low with resulting better current efficiency. Although nearly 100% current efficiency was obtained in this laboratory for several titrations of ferric ion with titanous ion by the use of the gold electrode with internal generation, other titrations were ruined by hydrogen evolution. The frequent cleaning and flaming treatment of the gold electrode, recommended by Arthur and Donahue to avoid such erratic behavior, was not convenient.

Preliminary experiments in generating titanous ion at a large mercury cathode indicated that Orange II could be titrated by the internal generation of titanous ion if the temperature of the titration was considerably below the boiling point. However, the rate of reaction of titanous ion with the dye was slowed down. On raising the temperature to near the boiling point, hydrolysis of titanium chloride solution and bubble formation at the mercury surface led to low current efficiencies.

De Ford (*3*) has pointed out that one of the advantages of external generation is that the electrolysis can be controlled without sample interference at the electrodes. Hence, the external generation method was considered a better approach, since titanous ion could be generated at room temperature and then delivered to the hot dye solution.

Preliminary experiments were carried out with a small mercury pool as the cathode in one arm of the De Ford T-tube cell. Current efficiencies of 98 to 99% were obtained. Mercury plated on a platinum wire coil and silver wire coil cathodes amalgamated with mercury, which were tried in order to obtain a larger electrode area, gave very low current efficiencies (54 to 75%). Pitts, De Ford, Martin, and Schmall (*6*) have used a single flow of electrolysis solution through a platinum gauze electrode contained in a stopcock for external generation of chlorine, bromine, and iodine. A somewhat similar cell design was used for generation of titanous ion, as this design was better suited for obtaining a larger mercury pool cathode surface and smaller hold-up volume. Furthermore, the single flow has further advantages over the T-tube arrangement in the saving of

Table II. Titration of Dyes

Dye	Titanous Ion, Coulometric Titration			Titration with 0.03N Titanous Chloride, % Dye
	% dye	No. of detns.	Standard deviation	
Orange II				
Direct titration	88.70	4	0.17	88.1
Indirect titration	88.96	4	0.20	88.5
Tartrazine, indirect titration	80.60	5	0.23	79.5
p-Aminoazobenzene HCl paste, indirect titration	44.4	1	..	44.4
Methyl violet, indirect titration	77.7	7	0.58	..

reagent, and the electric field can be evenly distributed directly above the mercury pool.

Titration of Dyes. Results of the coulometric titration of several (technical grade) dyes are shown in Table II along with the corresponding results obtained by ordinary standard titanous chloride titration (*5*) for some of these. A current of 260 ma. was used in order to obtain a 0.25-second visual sensitivity for the Orange II direct end point. Sample sizes were taken such that the titration required about 200 seconds. It was important to limit the time of titration, as the addition of too much titanium tetrachloride solution caused the titanium dioxide to precipitate, thus obscuring the end point.

The values in Table II indicate the kind of precision which can be obtained by the coulometric titration. In addition, there are given some values for these samples which were obtained in the authors' Control Laboratories under normal control laboratory conditions by titration with standard titanous chloride. In view of the generally accepted estimate of the accuracy and reproducibility of such determinations as given, for example, in Siggia (*9*), it may be concluded that there is no serious disagreement between the two methods.

The large excess of titanium chloride added by the coulometric method may have some effect on the buffer action. Evenson and Nagel (*4*) state that the function of the buffer (buffer catalyst) is not only to regulate the hydrogen ion concentration, but also to promote the reduction of the dye. The rate of reaction of titanous ion with the dye may be somewhat slower by the coulometric titration, as the titanic ion is about 20 times the titanous ion added—for example, the rate of reaction for the direct titration of tartrazine was slowed so that it was necessary to employ the indirect end point procedure. The titration of aminoazobenzene (oil-soluble) in alcohol-water solution was satisfactory; this dye is normally run by the indirect procedure.

ACKNOWLEDGMENT

The authors wish to express their appreciation to N. Howell Furman, Princeton University, and to G. L. Royer, for their interest and many helpful suggestions; to D. D. De Ford, Northwestern University, for prepublication information on the single arm cell design (*6*); and to G. E. Gerhardt and H. C. Lawrence, who constructed and assembled the electrical equipment.

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Received for review July 30, 1954. Accepted October 14, 1954.

Titration of Acids in Dimethylformamide Using High Frequency

JOHN A. DEAN and CARL CAIN, JR.
Department of Chemistry, University of Tennessee, Knoxville, Tenn.

The purpose of the investigation was to determine the applicability of a high frequency oscillator to the titration of acids in dimethylformamide. Sharp V-shaped titration curves are obtained for strong acids and the ammonium ion over the complete range of the sensitivity of the instrument which includes concentrations as small as 0.0001M. Less acute angles are obtained in the titration of acids of intermediate strength, although generally adequate end point discrimination is possible for acids whose pK_a values do not exceed 7 in water. The high frequency method should be a useful adjunct for titrations in dimethylformamide, particularly as only a very limited number of color indicators are available at present.

IT WAS the purpose of the present investigation to determine the applicability of a high frequency oscillometer to the titration of acids in dimethylformamide. Wagner and Kauffman (*6*) have reported a successful application of high frequency titrations to organic bases in glacial acetic acid, and Ishidate and Masui (*3*) have titrated a number of organic acids in a benzene-methanol mixture by high frequency.

Dimethylformamide has been used as a titration medium for a number of weak organic acids. Fritz (*2*) has recommended the use of the solvent for the titration of enols and imides and negatively substituted phenols. Vespe and Fritz (*5*) have determined many of the sulfa drugs as acids by titration in dimethylformamide. Fritz (*1*) has also suggested a procedure for the determination of salts of strong bases—i.e., their conjugate acids—in dimethylformamide.

The lack of a variety of suitable visual indicators, or adequate electrode systems for potentiometric titrations, is a major limitation to the use of dimethylformamide. The range of acid strengths which may be successfully titrated in the solvent has only been qualitatively estimated.

APPARATUS AND REAGENTS

The high frequency measurements were made with the Sargent Chemical oscillometer, Model V. The frequency of the instrument was approximately 4.89 megacycles. After the addition of each titration increment the instrument was brought back into resonance by adjusting appropriate capacitances in parallel with the titration cell (*4*). All subsequent references to capacitance readings or measurements in this paper refer to the high frequency capacitances as measured by this instrument.

The titrations were carried out in a standard cell supplied with the instrument. The annular space between the condenser plates had a volume of 50 ml. In all the titrations a volume considerably in excess of this amount was employed, making it possible to perform the titration without changing the volume of solution between the plates of the condenser.

A stirring motor was in position to be raised or lowered into the titration cell, which was fitted with a polyethylene cover through which the stirrer, inlet tube for nitrogen gas, and buret tip were inserted. Carbon dioxide was excluded from the system by directing a stream of nitrogen over the surface of the solution. A 10-ml. microburet, which could be read to the nearest 0.01 ml., was used for all titrations.

Dimethylformamide, technical grade, obtained from E. I. du Pont de Nemours & Co. was used without further purification. It contained approximately 0.03 meq. of acid impurity (presumably formic acid) per 100 ml. The presence of this impurity necessitated the neutralization of the solvent, or a blank correction.

All acids were c.p. grade, and when possible, those meeting ACS specifications were employed.

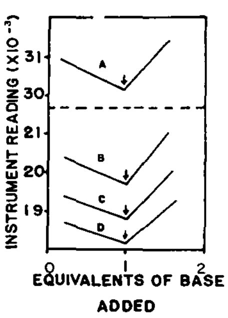

Figure 1. Effect of dilution on titration of strong acids

A. 0.006*M*
B. 0.0009*M*
C. 0.0005*M*
D. 0.00025*M*

EXPERIMENTAL

The following procedure was used for all high frequency titrations.

The instrument was allowed to warm up for 1 hour. Ninety-five milliliters of dimethylformamide were placed in the titration cell, followed by 5 ml. of the sample solution. An indicator was added, the titration assembly was lowered into place, and the nitrogen gas flow was adjusted. With the stirring motor running, resonance was established by adjustment of the calibrated condensers. The titration was carried out by adding approximately 0.5-ml. increments of the titrant, and recording the condenser reading required to re-establish resonance after each addition. Addition of the titrant was continued until the end point was reached, and seven or eight readings were obtained beyond it. The condenser readings were plotted against volume of titrant added; the intersection of the extrapolated straight-line portions of the plot was taken as the end point of the titration.

Standardization of Sodium Methoxide Solution. A sodium methoxide solution, approximately 0.1*N*, was prepared by dissolving 3 grams of sodium, previously washed in absolute methanol, in 50 ml. of absolute methanol, then diluting to 1 liter with a 4 to 1 mixture of benzene-methanol. The solution was standardized against benzoic acid, using thymol blue indicator, according to the procedure of Fritz (*1*). Standard 0.01*N* solution was prepared by proper dilution of the 0.1*N* solution. Standardization was also carried out in a benzene-methanol solvent mixture by high frequency titration (*3*). The 0.01*N* solution was used as the titrant in this investigation.

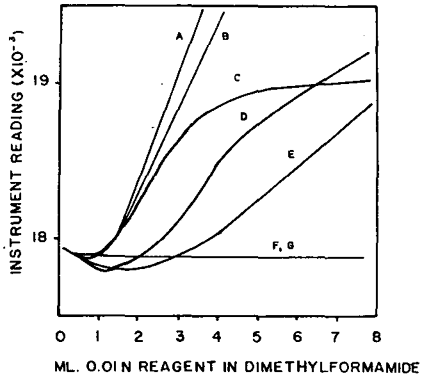

Figure 2. Instrument response curves

A. Perchloric acid
B. *p*-Toluenesulfonic acid
C. Salicylic acid
D. Sodium methoxide
E. Sulfuric acid
F, G. Formic acid, *n*-butylamine

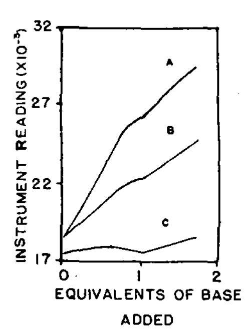

Figure 3. Effect of dilution on titration of intermediate strength acids

A. 0.0036*M*
B. 0.0022*M*
C. 0.0004*M*

TITRATION OF DISSOLVED ACIDS

High frequency titrations of acids of widely different pK_a values were attempted. The titration of strong acids with sodium methoxide solution produces in dimethylformamide curves which resemble the low frequency conductance curves for strong acids in water (Figure 1). The negative slope of the curve prior to the end point indicates the removal from solution of a highly capacitative ion, the protonated solvent ion, DMF.H$^+$. The removal of this ion decreases the capacitance and consequently the frequency of the oscillometer. A minimum was reached at the point in the titration curve where the DMF.H$^+$ ion was completely removed. Subsequent addition of sodium methoxide titrant produced an increase in the capacitance of the titration cell. The intersection obtained by extrapolation of the two branches of the titration curve results in a very acute angle at the end point. The difference in capacitance between the DMF.H$^+$ ion and the methoxide ion is small, as shown by the similar slopes of the two branches of the titration curve, and consequently, the indicated end point essentially coincides with the stoichiometric end point.

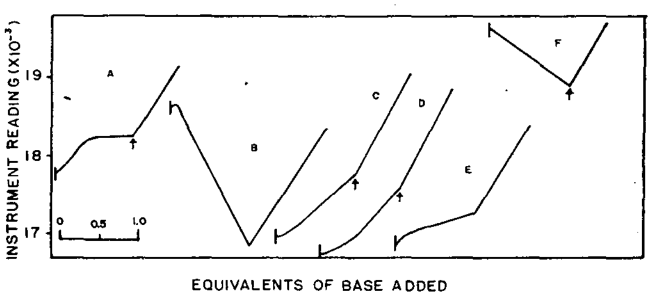

Figure 4. Titration curves of various acids

A. Salicylic acid
B. Potassium hydrogen phthalate
C. Benzoic acid
D. *o*-Nitrophenol
E. Boric acid
F. Ammonium ion

The instrument response curves for the DMF.H$^+$ ion and the methoxide ion (Figure 2) indicate that a maximum response would be obtained for the titration of strong acids over the complete range of the instrument's sensitivity, with the exception of concentrations less than 0.0001*M*. The curves obtained for the titration of very dilute solutions thus contain a break of the same magnitude as those obtained with the more concentrated solutions (Figure 1). These observations are contrary to the response observed with low frequency titration curves.

The response curve for salicylic acid (Figure 2) indicates that in low concentrations the proton exchange is virtually complete from acid to solvent, while in concentrations above 0.0005*M* its behavior is that of a slightly dissociated acid. This effect is shown by the titration curves in Figure 3.

In the titration of acids of intermediate strength, less acute angles were obtained from the intersection of the two branches of the titration curve. The results for salicylic acid (pK_a = 2.9), potassium acid phthalate (pK_a = 3.24), benzoic acid (pK_a = 4.20), *o*-nitrophenol (pK_a = 7.21), boric acid (pK_a = 9.24), and ammonium ion (pK_a = 9.24) are summarized in Figure 4. The small arrows appearing on the curves indicate the point at which thymol blue indicator (pK = 8.9) changed from yellow to blue. The horizontal scales (equivalents of base added) are the same, but the zero is shifted in each case to the right. Acids whose pK_a values in water exceed 4 are essentially nonionized in dimethylformamide, as is shown by their initially low capacitance and the nonexistence of any appreciable response curve (see curve *F* for formic acid in Figure 2). Except for concentrations less than 0.001*M*, the titration curves for these intermediate strength acids show increasing capacitance throughout the course of the titration. Prior to the end point of the titration the shape of the curve is determined by the capacitance of the sodium ion (from the sodium methoxide used as titrant) and the conjugate base ion of the respective acid. Following the end point, the increased slope of the titration curve is a result of the addition of excess sodium methoxide to the solution, exactly as for the titration of strong acids.

The difference in slope of the two branches of the titration curve is a measure of the difference in capacitance of the conjugate base ion and of the methoxide ion. This difference will determine whether or not a satisfactory end point can be secured upon extrapolation of the two branches. For example, in the titration of benzoic acid the capacitance of the benzoate ion and that of the methoxide ion are approximately equal. The change in capacitance during the release of the benzoate ion practically equals the change resulting from the addition of excess methoxide ion to the solution, and consequently, the titration curve exhibits only a small break at the end point.

Removal of ionic species from solution by precipitation or the formation of a volatile product improves the recognition of the end point considerably. The removal of high capacitance ions through evolution of gaseous ammonia during the neutralization of NH_4^+ effectively lowered the capacitance of the solution until the end point was attained, after which the addition of excess methoxide caused a sudden rise. The resulting titration curve resembles those obtained for the titration of strong acids. Similarly, the precipitation of sodium potassium phthalate during the titration of potassium acid phthalate is an advantage, although equilibrium was established very slowly after each addition of titrant.

The titration of phenol (pK_a = 9.9) was attempted but no satisfactory end point was achieved.

DISCUSSION

Results obtained from the investigation of the stoichiometry of the titrations indicates sufficient accuracy for the application of the high frequency method to titration of acids in dimethylformamide. Table I shows that the results of separate determinations are consistent within the limits of precision with which the titrations were performed. Proper calibration of volumetric ware and the control of temperature would considerably enhance the accuracy of the determinations.

In the use of thymol blue indicator, the solvent was neutralized to the base color of the indicator before the addition of the acid sample. A blank correction was obtained from the high frequency determinations from a graph of the end points obtained from the titration of a series of acid sample dilutions. These were extrapolated to zero concentration.

Only in the case of the strong acids, acids whose pK_a values are less than 3 in water, and the ammonium ion, are the indicator and instrument end points in agreement. For the other strength acids the indicator color change occurred after the instrument end point, indeed if a color change was observed at all. In the titration of boric acid, thymol blue exhibited its base color throughout the titration. Since a range of suitable indicators is presently not available for dimethylformamide, the high frequency method becomes a useful adjunct to the titration of acids in dimethylformamide.

Table I. Analysis of Acids

Acid	Type	Purity, %
Ammonium bromide	High frequency	99.7 99.2 101.3
	Indicator	100.4 100.7 100.7
Ammonium iodide	High frequency	100.6 100.3 99.2
	Indicator	99.2 100.0 100.2
Salicylic acid	High frequency	101.0 100.2
	Indicator	99.7 101.0 100.2
o-Nitrophenol	High frequency	97.9
	Indicator	97.9

The use of dimethylformamide as a solvent appears to increase to a slight degree the acid strength of compounds which are incompletely ionized in water. However, the principal usefulness of the solvent is in the dissolution of materials which are only slightly soluble in water. With sodium methoxide as titrant the minimum acid strength which gives a distinguishable break at the end point appears to be an acid with a pK_a value of approximately 7, unless one of the titration products is removed from the reaction theater. *n*-Butylamine is also a suitable titrant for the stronger acids. Extensive solvolysis in the vicinity of the end point precludes its use for acids weaker than formic acid or benzoic acid.

Acids of differing strengths sometimes may be determined by means of successive titrations whereby two breaks are obtained in the titration curve. Often special procedures are necessary.

ACKNOWLEDGMENT

The authors wish to express their appreciation to the E. H. Sargent and Co. for the loan of the high frequency oscillometer used in this work.

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Received for review June 26, 1954. Accepted November 9, 1954. Presented at the Southeastern Regional Meeting of the American Chemical Society, Birmingham, Ala., 1954. Contribution No. 137 of the Department of Chemistry, University of Tennessee. Abstracted from the master of science thesis of Carl Cain, Jr., March 1954.

Wet Carbon Combustion—Correction

In the article on "Wet Carbon Combustion and Some of Its Applications" [Anal. Chem., **26**, 1707 (1954)] footnotes [b] and [c] of Table I should refer to potassium iodate instead of potassium chlorate.

Donald D. Van Slyke

Amperometric Titrations with Hypochlorite in the Presence of Bromide

H. A. LAITINEN and DALE E. WOERNER[1]

Noyes Chemical Laboratory, University of Illinois, Urbana, Ill.

Because of the instability of hypobromite, it is advantageous to titrate with hypochlorite in the presence of bromide. Amperometric titrations of arsenite and of ammonia with hypochlorite are comparable in sensitivity and accuracy with hypobromite titrations. Thiocyanate may be titrated with an accuracy of 0.5 to 1% in concentrations down to $10^{-4}M$, with increasing titration error at increasing dilution. Attempts to titrate several other reductants were unsuccessful because of the slowness of their reaction with hypobromite.

KOLTHOFF, Stricks, and Morren (*5*) have recently employed sodium hypobromite as a reagent in the amperometric titration of ammonia, and have used the arsenite-hypobromite titration for standardization. This work came to the authors' attention during the present investigation, in which similar titrations are carried out using hypochlorite as the reagent, in the presence of bromide. Because of the advantage of greater stability of standard solutions of hypochlorite (*4*), it appears worth while to describe the results obtained with this reagent.

Hypochlorite has been used as a reagent for several titrations using cochineal as indicator (*1*), and in potentiometric titrations (*8, 9, 12*), and has been determined amperometrically (*2, 6, 7*). Hypobromite has been used as a reagent in several potentiometric titrations (*3, 8–11*).

EXPERIMENTAL

Reagents. Calcium hypochlorite solutions were prepared by a method similar to that recommended by Kolthoff and Stenger (*4*), and standardized in the usual iodometric manner. A $0.05M$ solution, stored in a dark bottle at room temperature decreased in molarity at the rate of about 0.2% in 20 days. In contrast, Kolthoff, Stricks, and Morren (*5*) recommend a daily standardization of $0.06M$ hypobromite solution stored in the dark and under refrigeration.

Arsenite solutions were prepared from primary standard grade arsenious oxide, and ammonia-free water was prepared by redistillation of conductivity water by appropriate methods (*5*).

Rotating Platinum Electrode. The rotating microelectrode consisted of a 5.5-mm. length of 0.4-mm. diameter platinum wire projecting radially from the bottom of a length of 6-mm. soft glass tubing. Contact was made by a copper wire dipping into the top end of a mercury column filling the electrode tube. The electrode was driven at 1100 r.p.m. by a cone-drive motor.

After each titration, the electrode was cleaned of any adhering calcium carbonate by treating it with dilute nitric acid, rinsing it with conductivity water, and allowing it to dry in the air.

CURRENT-VOLTAGE CURVES

The current-voltage curves of hypobromite and hypochlorite in the presence of air are shown in Figure 1. It is apparent that hypobromite diffusion currents can be readily observed in the presence of air, while the oxygen reduction occurs at potentials corresponding to the diffusion current region of hypochlorite.

To test the proportionality of diffusion current of hypobromite, prepared in situ, with concentration, a solution of 1 gram of sodium bicarbonate and 2 grams of potassium bromide in 50 ml. of conductivity water ($0.24M$ bicarbonate and $0.34M$ bromide) was treated with successive increments of $0.022M$ hypochlorite solution in a beaker open to the air, and stirred with a magnetic stirrer after each addition. The stirrer was turned off during current measurements. Diffusion currents, measured at +0.2 volt (*vs.* S.C.E.), using an external calomel electrode with a saturated potassium chloride salt bridge, proved to be proportional to concentration up to $5 \times 10^{-4}M$ hypochlorite. Dissolved oxygen did not interfere at this potential.

An attempt was made to observe a kinetic current due to the transformation

$$OCl^- + Br^- \rightarrow OBr^- + Cl^- \tag{1}$$

at the electrode surface. At a potential of 0.6 volt, the current-voltage curve of hypobromite is approaching its diffusion-limited plateau, while hypochlorite gives only a small current. If Reaction 1 is very rapid, a solution containing only a trace of bromide would yield the same current as one containing a large amount of bromide. Actually, a $10^{-3}M$ hypochlorite solution in $0.24M$ bicarbonate gave a current of 0.88 μa., while a similar solution treated with enough bromide to yield $10^{-5}M$ hypobromite according to Reaction 1 gave 0.96 μa. The increment in current was actually less than that expected for a diffusion-controlled current of $10^{-5}M$ hypobromite, and showed no evidence of a kinetic current. The rate of Reaction 1, at $10^{-5}M$ concentration of bromide and $10^{-3}M$ hypochlorite is apparently rather slow, even though in the presence of excess bromide it proceeds rapidly by ordinary standards (a diffusion current of hypobromite is instantly observed after addition of hypochlorite and stirring).

AMPEROMETRIC TITRATIONS

A potential of 0.2 volt *vs.* S.C.E. was applied in all titrations unless otherwise noted, using an external saturated calomel electrode and potassium chloride salt bridge. The Sargent Model III manual polarograph and the Fisher Elecdropode were used.

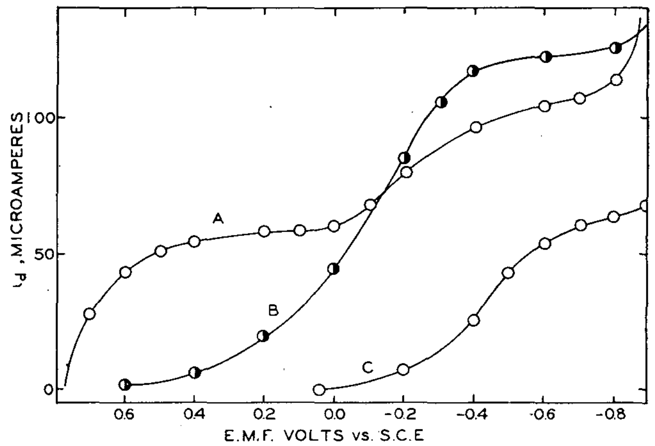

Figure 1. Polarograms of $4.4 \times 10^{-4}M$ hypohalite in $0.24M$ sodium bicarbonate saturated with air

A. Hypobromite prepared in situ in presence of $0.34M$ KBr
B. Hypochlorite
C. Residual current

For the most part, the titrations were performed in a shallow crystallizing dish, 80 mm. in diameter, with a small glass-enclosed magnetic stirring bar for mixing.

Arsenite Titration. Accurately standardized $0.03785M$ hypochlorite solution was used to titrate a conductivity water solution containing 25.00 ml. of $0.1202N$ arsenious oxide plus 10 ml. of a solution 10% in potassium bromide and 5% in sodium bicarbonate. This solution produced an unsteady current of the order of −1 μa. (anodic) at +0.2 volt. The anodic current was evidently due to arsenite, since mixtures of bromide and

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[1] Present address, Hanover College, Hanover, Ind.

bicarbonate gave currents of less than 0.1 μa. As hypochlorite was slowly added, the current approached zero (less than 0.1 μa), and remained there until after the end point. A typical curve is shown in Figure 2. The equivalence point was calculated to be 39.70 ml. Four titrations yielded graphical end points of 39.65, 39.86, 39.69 and 39.73 ml. No corrections for blanks were made.

To test the end point in a dilute solution, standard solutions which were 0.3m*M* in arsenite were titrated with standard 0.004*M* hypochlorite. The titrant was added from a 5-ml. semimicroburet with marked divisions of 0.01 ml. Blank titrations were run in all cases on solutions made up at the same time, and were similar except for the substitution of a volume of ammonia-free water equal to that of the arsenite (which was prepared from ammonia-free water). The results are given in Table I. One titration, which yielded a titration curve of very abnormally low slope probably because of an accidental high resistance, also gave an error of +11.4%, and was omitted.

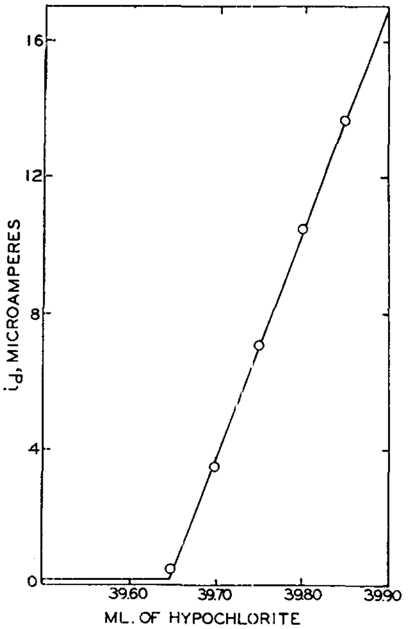

Figure 2. Amperometric titration of 25.00 ml. of 0.1202*N* sodium arsenite in 0.24*M* potassium bromide and 0.17*M* sodium bicarbonate with 0.07571*N* calcium hypochlorite

Blanks made up at the same time and from the same solutions agreed to within an average of 0.005 ml. of 0.005*M* hypochlorite, although over a period of time they showed considerable fluctuation (Table I).

Ammonia Titration. Reagent grade ammonium sulfate was used to prepare a 0.05*M* standard solution, and more dilute solutions were made by dilution with ammonia-free water.

Titrations were carried out using initial concentrations of 1.25 × $10^{-3}M$ bromide and 0.15*M* bicarbonate. In agreement with Kolthoff and coworkers (*5*) it was found that with a potential of +0.2 volt *vs.* S.C.E., a current of as much as 3 μa. was observed during the titration because of the slowness of the reaction between hypobromite and ammonia. The titration plot showed strict linearity beyond the end point.

Using conductivity water for all solutions, large and erratic blanks were observed. Accordingly, ammonia-free water was used, with the results in Table II.

It is concluded that the reaction between hypobromite prepared in situ and ammonia is satisfactory in concentrations of ammonia as low as 2.5 × $10^{-5}M$, with accuracy and precision being strongly sacrificed below $10^{-4}M$. This method is comparable in accuracy with that of Kolthoff, Stricks, and Morren (*4*), but has the advantage of using a more stable reagent.

Thiocyanate Titration. Thiocyanate is oxidized in alkaline media by hypobromite according to Equation 2 (*9*):

$$SCN^- + 4OBr^- + 2OH^- = SO_4^{--} + CNO^- + 4Br^- + H_2O \quad (2)$$

Table I. Titration of Arsenic as Arsenite with Hypochlorite in the Presence of Bromide

Arsenic, 0.936 mg., arsenite, 5 ml. of 0.0025*M* in total volume of 35 to 40 ml. with 0.004*M* hypochlorite, in 0.15*M* $NaHCO_3$, 1.25 × $10^{-3}M$ KBr.

Molarity[a], OCl^- × 10^3	Vol., OCl^-, Ml.	Blank, Ml.	Net Vol., OCl^-, Ml.	Theor. Vol., OCl, Ml.	Error, %
4.560	2.830	0.099	2.731	2.741	−0.4
4.520	2.882	0.083	2.799	2.766	+1.2
4.556	2.841	0.051	2.790	2.744	+1.7
4.556	2.847	0.044	2.803	2.744	+2.2

[a] Standardized iodometrically against thiosulfate.

Table II. Titrations of Ammonia in 0.15*M* Sodium Bicarbonate and 1.25 × $10^{-3}M$ Potassium Bromide, Initial Volume 40 Ml.

NH_3, Mg.	Molarity, OCl^- × 10^3	Blank, Ml.	Ml. OCl^-, Corr. for Blank	Ml. OCl^-, Theor.	Error, %
0.3406	11.36	0.030	2.672	2.641	1.2
0.1703	4.56	0.050	3.363	3.289	2.2
0.0851	4.56	0.082	1.671	1.645	1.6
0.0851	4.52	0.059	1.673	1.659	0.8
0.0341	1.136	0.327	2.730	2.641	3.4
0.0170	4.56	0.052	0.361	0.329	10.0
0.0170	1.136	0.238	1.404	1.320	6.4

Preliminary titrations of 25-ml. samples of 0.01250*M* thiocyanate, prepared from reagent grade potassium thiocyanate taken as a primary standard, indicated errors of −0.3 to +0.1% when 0.05*M* hypochlorite was used as the reagent, and the initial solution was 0.24*M* in bromide and 0.17*M* in bicarbonate. It was found that the current before the end point remained below 0.2 μa., indicating a reasonably rapid reaction between thiocyanate and hypobromite. After the end point, the current increased to a maximum value after each addition, after which it decreased slowly. The maximum current was taken as the reading.

A titration performed in a Clark and Lubs borate buffer of pH 9 gave results in good agreement with those performed in the bicarbonate solutions, but the current changed more slowly with time after the end point.

A brief investigation was made of titration of thiocyanate in concentration as low as 5 × $10^{-5}M$, under conditions identical with those described above for the ammonia titrations. However, since conductivity water rather than ammonia-free water was used in preparing the thiocyanate solution, blanks were run with corresponding volumes of conductivity water substituted for the thiocyanate. Some results are listed in Table III. Errors of the order of 0.5 to 1% may be expected in concentrations down to $10^{-4}M$, with increasing titration error at increasing dilution.

Table III. Titrations of Thiocyanate in 0.15*M* Sodium Bicarbonate and 1.25 × $10^{-3}M$ Potassium Bromide

(Initial volume 40 to 45 ml.)

SCN^-, Mg.	Molarity, OCl^- × 10^3	Blank, Ml.	Ml. OCl^-, Corr. for Blank	Ml. OCl^-, Theor.	Error, %
1.818	45.54	0.031	2.744	2.749	−0.2
0.363	11.36	0.102	2.218	2.201	+0.8
0.109	4.554	0.323	1.688	1.648	+2.4

Attempted Titrations of Other Reductants. Exploratory work was done with a number of other reductants to determine the feasibility of amperometric determinations.

Twenty-five milliliters of 0.10*N* reductant were mixed with 10 ml. of a base solution which was 10% in potassium bromide and 5% in sodium bicarbonate. The solution was titrated with standard 0.075 or 0.09*N* hypochlorite, and current readings were taken at a potential of +0.2 volt *vs.* S.C.E.

With urea, nitrite, sulfite, pyrosulfite, sulfide, hypophosphate, cyanide, and hydrogen peroxide, the reaction was found to be too slow for a feasible titration. In general, a current due to hypobromite was observed far before an equivalent amount of hypochlorite had been added. If an end point could be observed at all, it came far too early. With formate, methanol, ethyl alcohol, or *tert*-butyl hydroperoxide, no oxidation occurred under the conditions of the titration, since the first additions of reagent gave proportionate increases of current.

Under more strongly alkaline conditions, hypobromite reactions might be expected to be more rapid and stoichiometric. However, at higher pH values hypobromite yields no diffusion current at potentials more positive than the region corresponding to oxygen reduction. Such titrations, particularly in dilute solutions, would therefore have to be carried out in air-free solutions.

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RECEIVED for review June 23, 1954. Accepted November 15, 1954.

Infrared Method for Determination of *o*-, *m*-, and *p*-Mononitrotoluene and 2,4-Dinitrotoluene in Mixtures

FRANK PRISTERA and MICHAEL HALIK

Picatinny Arsenal, Dover, N. J.

For the efficient study of the nitration of toluene an exact knowledge of the nitration products formed in the first stage of nitration is necessary. Such products consist essentially of *o*-, *m*-, and *p*-mononitrotoluene with small amounts of dinitrotoluene. For the analysis of such products, as a mixture, an infrared spectrophotometric method has been developed which is fast, rather accurate and precise, and applicable over very wide ranges of concentration for each ingredient. The method involves making absorbance measurements of a 20% solution of the sample in cyclohexane at the peaks of the 8.49-, 8.70-, 9.39-, and 12.49-micron bands which are those of *p*- and *o*-nitrotoluene, 2,4-dinitrotoluene, and *m*-nitrotoluene. Using the method of successive approximations, the measured absorbances, after beng corrected for solvent and cell absorbance, were calculated to per cent of the ingredients. Since this method was first developed, it has been extended to include also 2,6-dinitrotoluene, which is determined by measuring a 25% benzene solution of the sample at the peak of the 11.22-micron band.

IN THE manufacture of trinitrotoluene by the nitration of toluene, *o*-, *m*-, and *p*-mononitrotoluene are produced in the first stage of nitration. The meta isomer on further nitration yields trinitro isomers other than thed esired 2,4,6-trinitrotoluene. These isomers are generally undesirable as they cause exudation in trinitrotoluene and therefore must be removed. A method of nitration that would produce the least amount of *m*-nitrotoluene, at the first stage of nitration, would therefore be desirable. The efficient development of such a nitration method requires a method of analysis of mixtures of *o*-, *m*-, and *p*-nitrotoluene and also 2,4-dinitrotoluene, which is probably present to some extent in the first stage of nitration of toluene. Such a method of analysis, in addition, can be advantageously used to study nitration kinetics which in turn can be used for the establishment of an efficient nitration method.

As no method of analysis of mixtures of *o*-, *m*-, and *p*-nitrotoluene, and 2,4-dinitrotoluene has been found in the literature, it was considered desirable to develop one. Considering the successful use of infrared spectroscopy in the analysis of trinitrotoluene isomeric mixtures (*1*), it was considered advisable to investigate the use of this tool in the development of a method for the analysis of mixtures of *o*-, *m*-, and *p*-nitrotoluene and 2,4-dinitrotoluene. An examination of the infrared spectrograms (see Figures 1 through 4) of the ingredients confirmed the feasibility of using infrared spectroscopy for the analysis of such a mixture.

SUMMARY OF METHOD AND RESULTS ON SYNTHETICS

The developed method involves preparation of cyclohexane solutions of the sample of suitable concentration; measurement of the prepared solutions at the peaks of absorption bands occurring at about 8.70 for *o*-nitrotoluene, 12.49 microns for *m*-nitrotoluene, 8.49 microns for *p*-nitrotoluene, and 9.39 microns for 2,4-dinitrotoluene; and relating the absorbances to concentrations from previously established relationships (absorptivities) using the method of successive approximations for calculations.

The developed method was applied to seven synthetic samples containing various amounts of *o*-, *m*-, and *p*-nitrotoluene and 2,4-dinitrotoluene. The results obtained are listed and treated statistically in Table I. A summary of the statistical values is shown in Table II.

Since the method was first developed it has been extended to include 2,6-dinitrotoluene, which is determined by measuring a 25% benzene solution of the sample at the peak of the 11.22-micron band. In this connection the following absorptivity data supplement Tables III and IV. The average absorptivities of 2,6-dinitrotoluene at the peaks of the 8.49-, 8.70-, 9.39-, 11.22-, and 12.49-micron bands are, respectively, 0.36, 0.53, 0.29, 12.5, and 0.49. The average absorptivities of *o*-, *m*-, and *p*-nitrotoluene and 2,4-dinitrotoluene at 11.22 microns are, respectively, 0.18, 0.60, 0.18, and 0.55. Also the cell absorbance at 11.22 microns is 0.04, and the absorptivity of benzene is 0.06 per 0.10 mm. The infrared spectrograms of *o*-, *m*-, and *p*-nitrotoluene, 2,4-, 2,6-dinitrotoluene, and the solvents cyclohexane and benzene are included in this report (see Figures 1 through 7).

EXPERIMENTAL PROCEDURE

Instrumentation. The infrared work was performed using a Perkin-Elmer Double Beam infrared spectrophotometer (Model

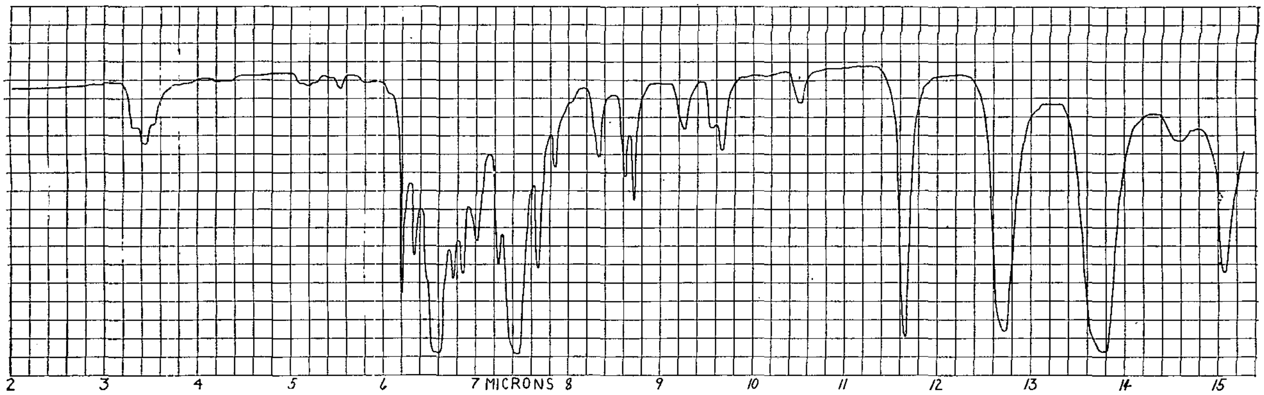

Figure 1. Infrared spectrogram of *o*-nitrotoluene

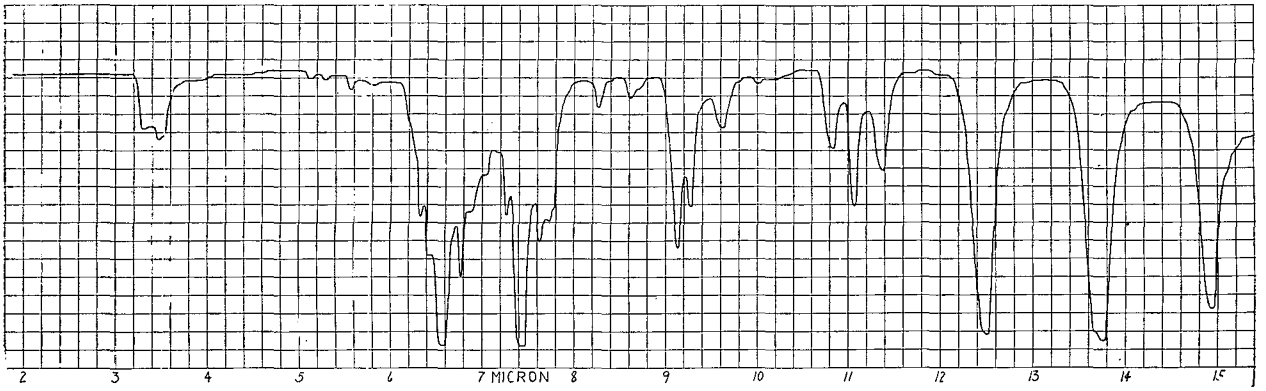

Figure 2. Infrared spectrogram of *m*-nitrotoluene

Table I. Values Obtained on Synthetics of *o*-, *m*-, and *p*-Nitrotoluene and 2,4-Dinitrotoluene

	Added, %	Found, %					Absolute Error[a], %	Relative Error[b], %	Standard Deviation[c]	Coefficient of Variation[d]
		a	*b*	*c*	*d*	Av.				
Sample I										
o-Nitrotoluene	85.74	86.55	87.20	87.20	87.20	87.04	1.30	1.49	1.03	1.18
m-Nitrotoluene	7.51	8.15	7.80	7.80	7.80	7.89	0.38	4.82	0.17	2.16
p-Nitrotoluene	2.01	3.58	3.40	3.25	2.90	3.28	1.27	38.7	0.25	7.62
2,4-Dinitrotoluene	4.74	4.40	4.45	4.50	4.55	4.48	−0.26	−5.80	0.06	1.34
Sample II										
o-Nitrotoluene	49.83	48.55	53.90	50.50	52.50	51.36	1.53	2.98	2.38	4.63
m-Nitrotoluene	8.14	8.80	8.60	8.80	8.80	8.75	0.61	6.97	0.22	2.51
p-Nitrotoluene	39.86	37.80	38.40	37.60	37.20	37.75	−2.11	−5.59	0.49	1.30
2,4-Dinitrotoluene	2.17	2.30	2.50	3.00	2.85	2.66	0.49	18.4	0.32	12.0
Sample III										
o-Nitrotoluene	59.69	57.70	59.75	62.45	57.70	59.40	−0.29	−0.49	2.25	3.79
m-Nitrotoluene	20.04	19.35	19.45	19.25	19.35	19.35	−0.69	−3.57	0.08	0.42
p-Nitrotoluene	15.02	15.05	15.17	14.75	14.90	14.97	−0.05	−0.33	0.18	1.20
2,4-Dinitrotoluene	5.24	5.35	5.45	5.02	5.00	5.21	−0.03	−0.58	0.23	4.39
Sample IV										
o-Nitrotoluene	64.26	61.75	66.45	63.05	66.45	64.42	0.16	0.25	2.40	3.72
m-Nitrotoluene	30.56	32.00	32.40	32.50	32.15	32.26	1.70	5.27	0.23	0.72
p-Nitrotoluene	2.04	3.15	2.52	3.33	2.98	3.00	0.96	32.0	0.35	11.6
2,4-Dinitrotoluene	3.14	3.85	2.99	3.91	2.97	3.43	0.29	8.45	0.52	15.2
Sample V										
o-Nitrotoluene	54.53	56.56	57.24	58.26	54.87	56.73	2.20	3.88	1.43	2.52
m-Nitrotoluene	1.01	0.96	1.00	0.93	1.09	0.99	−0.02	−2.02	0.07	7.10
p-Nitrotoluene	39.65	37.87	40.30	40.00	41.09	39.81	0.16	0.40	1.37	3.45
2,4-Dinitrotoluene	4.81	4.78	4.85	4.94	5.08	4.91	0.10	2.04	0.13	2.65
Sample VI										
o-Nitrotoluene	58.71	58.64	60.34	60.34	61.03	60.09	1.38	2.30	1.02	1.70
m-Nitrotoluene	3.94	3.74	4.20	4.27	4.25	4.11	0.17	4.14	0.39	9.49
p-Nitrotoluene	35.00	33.83	33.96	36.02	35.95	34.94	−0.06	−0.17	1.21	3.46
2,4-Dinitrotoluene	2.35	3.03	1.95	2.24	2.18	2.35	0.00	0.00	0.47	20.0
Sample VII										
o-Nitrotoluene	30.02	31.90	30.89	32.59	31.90	31.82	1.80	5.66	0.70	2.20
m-Nitrotoluene	0.63	0.56	0.52	0.49	0.52	0.52	−0.11	−21.2	0.03	5.78
p-Nitrotoluene	60.05	57.06	58.40	58.40	57.40	57.81	−2.24	−3.88	0.69	1.19
2,4-Dinitrotoluene	9.30	10.14	9.82	9.59	9.74	9.82	0.52	5.29	0.23	2.36

[a] Absolute error = av. found − av. added.

[b] Relative error $= \dfrac{\text{absolute error} \times 100}{\text{av. added}}$.

[c] Standard deviation $= \sqrt{\dfrac{(X_i - \overline{X})^2}{n - 1}}$

where X_i = individual observation, $\overline{X}$ = av. found, and n = No. of observations.

[d] Coefficient of variation $= \dfrac{\text{standard deviation} \times 100}{\text{av. found}}$

21) in accordance with the instruction manual for the instrument. The instrument was fitted with an absorbance scale in addition to the transmittance scale provided with the instrument, so that absorbance readings could be taken directly from the instrument rather than be calculated from the transmittance readings. The instrument was calibrated (*2*) and found to be within 0.03 micron at the upper and lower limits of the rock salt regions and within 0.02 micron in the center region. The absorbance measurements were made using a single cell calibrated by the interference fringes method (*4*) and found to be 0.128 mm. Sodium chloride optics were used throughout.

Solvent. Cyclohexane, treated with anhydrous sodium sulfate and sodium chloride to minimize any solvent action on the sodium chloride cells, was the only solvent used.

Infrared Spectrograms. The infrared spectrograms of *o*- and *m*-nitrotoluene (liquids) were obtained on capillary films. The spectrograms of *p*-nitrotoluene and 2,4-dinitrotoluene (solids) were obtained by placing a few milligrams of the ingredients between two sodium chloride plates in a demountable cell. The cell assembly was then heated to produce a capillary film of the melt, which, after cooling, produced a well defined spectrogram.

Quantitative Method of Analysis. DEFINITIONS (*5*). Transmittance (T) = ratio of radiant power transmitted by sample to radiant power incident on it.

Absorbance (A) = logarithm to the base 10 of the reciprocal of the transmittance.

Absorptivity (a) = ratio of the absorbance to the product of concentration and length of optical path. (If only one cell is used for all the work, the path length is constant and can be left out of all the calculations without introducing any errors.)

DETERMINATION OF CELL ABSORBANCE, ABSORPTIVITY OF INGREDIENTS AND CYCLOHEXANE. The measurements were made

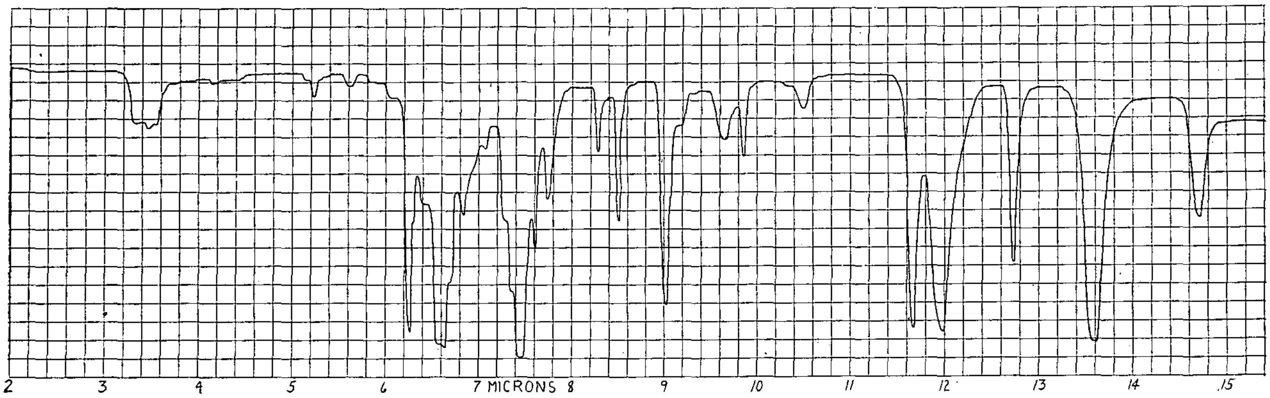

Figure 3. Infrared spectrogram of *p*-nitrotoluene

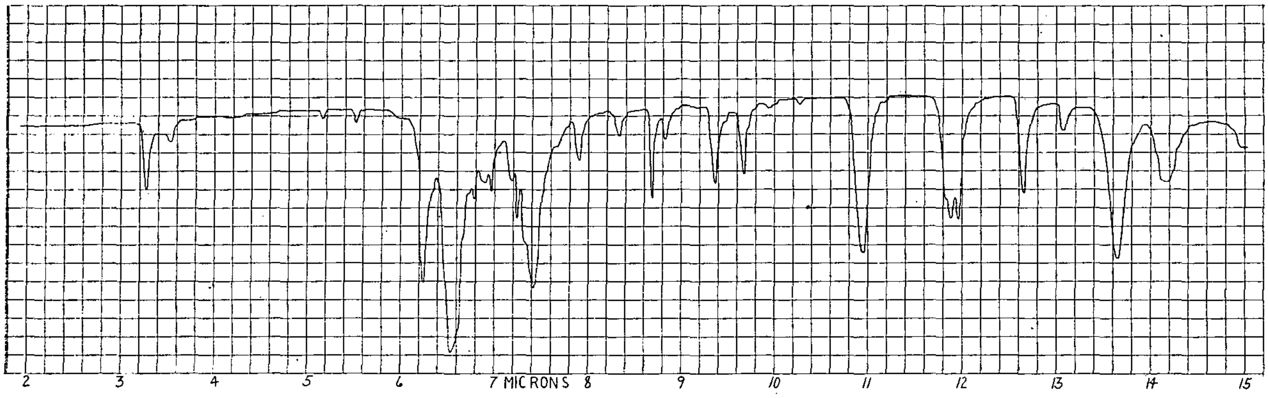

Figure 4. Infrared spectrogram of 2,4-dinitrotoluene

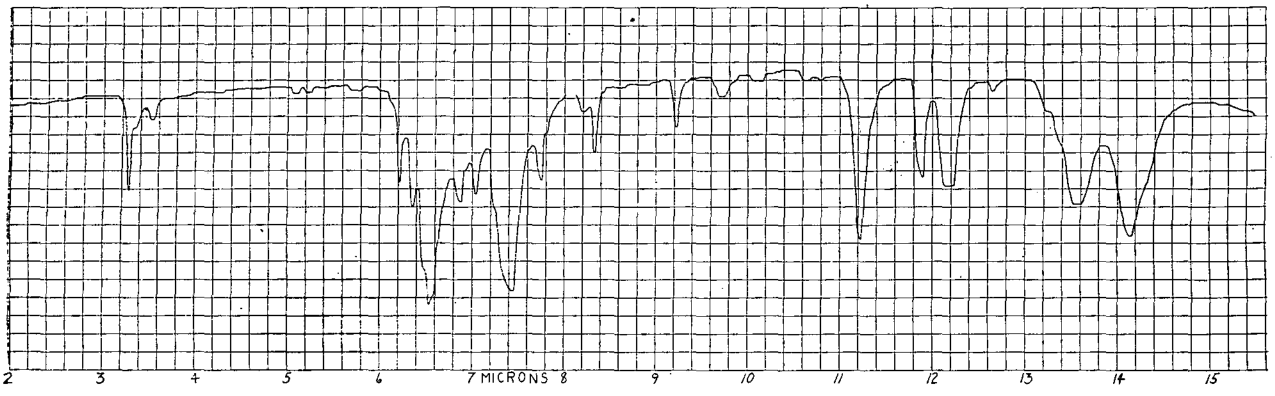

Figure 5. Infrared spectrogram of 2,6-dinitrotoluene

Table II. Summary of Results Obtained on Synthetics of *o*-, *m*-, and *p*-Nitrotoluene and 2,4-Dinitrotoluene

	o-MNT	*m*-MNT	*p*-MNT	2,4-DNT
Concentration range, %	30.02 to 85.74	0.63 to 30.56	2.01 to 60.05	2.17 to 9.30
Absolute error, %	−0.29 to +2.20	−0.69 to +1.70	−2.24 to +1.27	−0.26 to +0.52
Relative error, %	−0.49 to +5.66	−21.2 to +6.97	−5.59 to +38.7	−5.80 to +18.4
Standard deviation	0.70 to 2.40	0.03 to 0.39	0.18 to 1.37	0.06 to 0.52
Coefficient of variation	1.18 to 4.63	0.42 to 9.49	1.19 to 11.6	1.34 to 20.0

at the peak of the bands occurring at about the following points:

o-Nitrotoluene	8.70 microns
m-Nitrotoluene	12.49 microns
p-Nitrotoluene	8.49 microns
2,4-Dinitrotoluene	9.39 microns

Each absorbance reading was obtained by scanning very slowly from about 0.05 micron below to about 0.05 micron above the designated points to ensure measurement of maximum absorbance of the respective bands.

Two new, clear cells of about 0.1 and 0.5 mm. were calibrated and found to be 0.128 and 0.525 mm., respectively. The cells were filled with cyclohexane and the total absorbance of cells with solvent was measured at the four points mentioned above. The absorptivity of cyclohexane and cell absorbance were calculated as follows:

$$\text{Absorptivity of cyclohexane, } a = \frac{(A_{0.525} - A_{0.128})\,0.128}{0.525 - 0.128}$$

Since all the measurements were made using the same 0.128-mm. cell, all the calculations were simplified by calculating the absorptivity of cyclohexane on a 0.128-mm. basis, thereby eliminating the cell thickness from subsequent calculations.

$$\text{Cell absorbance of 0.128-mm. cell} = A_{0.128} - a$$

where $A_{0.525}$ and $A_{0.128}$ are, respectively, the absorbance of the 0.5- and 0.1-mm. cells filled with cyclohexane.

The values obtained for the absorptivity of cyclohexane and the absorbance of the 0.128-mm. cell at the above mentioned four points are given in Table III.

The absorptivities of the components at the points corresponding to the maximum absorption of the measured bands were determined by measuring the absorbance of known solutions of the different components ranging in concentrations from 0.50 to 20%. The absorptivity of the components at each point for each solution was calculated as follows (see Table IV for values):

$$\text{Absorptivity} = \frac{A_{\text{total}} - A_{\text{cell}} - A_{\text{solvent}}}{\text{Per cent of material in solution expressed as a decimal}}$$

where

A_{total} = total absorbance of cell filled with cyclohexane solution of ingredient

A_{cell} = absorbance of cell at the particular wave length (see Table III)

Table III. Absorptivity of Cyclohexane and Absorbance of 0.128-Mm. Cell

Wave length, microns	8.49	8.70	9.39	12.49
Absorptivity of cyclohexane (absorbance per 0.128 mm.)[a]	0.045	0.057	0.079	0.036
Absorbance of 0.128-mm. cell	0.045	0.045	0.045	0.044

[a] All measurements were made using same 0.128-mm. cell; calculations were simplified by omitting cell thickness, keeping in mind that absorptivity represents 0.128-mm. thickness.

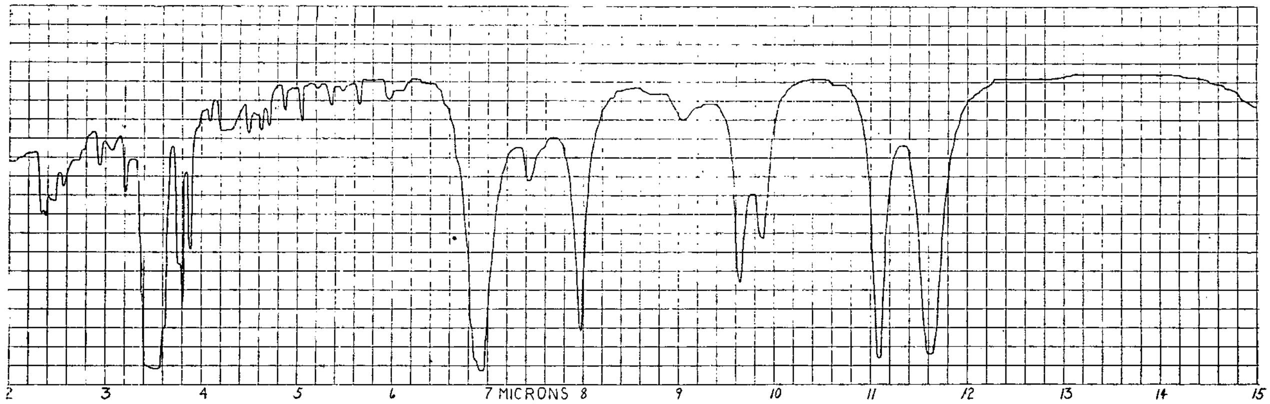

Figure 6. Infrared spectrogram of cyclohexane

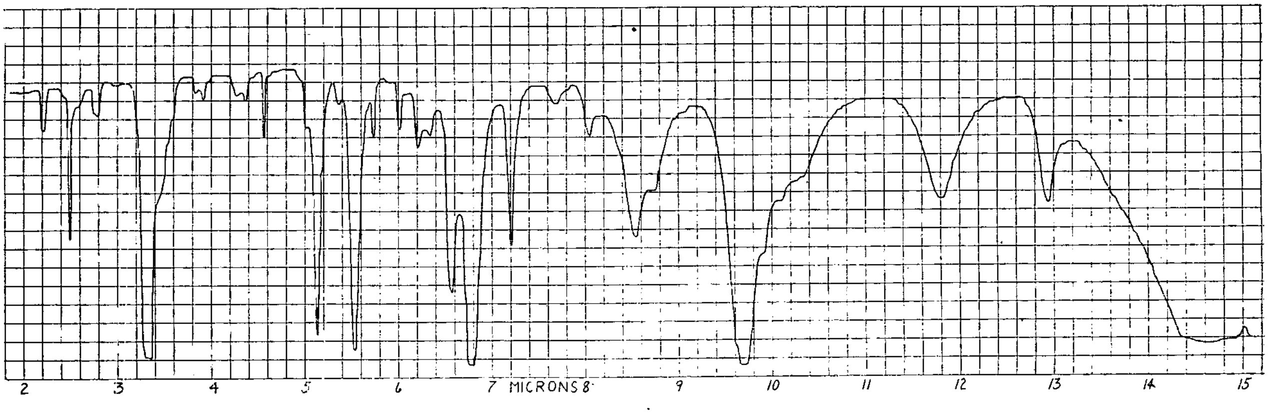

Figure 7. Infrared spectrogram of benzene

Table IV. Absorptivities of *o*-, *m*-, and *p*-Nitrotoluene and 2,4-Dinitrotoluene

(Absorbance per 0.128 mm.[a])

Absorptivity, %	*o*-Nitrotoluene				*m*-Nitrotoluene				*p*-Nitrotoluene				2,4-Dinitrotoluene			
	8.49	8.70	9.39	12.49	8.49	8.70	9.39	12.49	8.49	8.70	9.39	12.49	8.49	8.70	9.39	12.49
	Wave Length, Microns															
0.50	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3.92	..
0.75	..	..	..	..	..	..	..	..	..	..	..	..	0.31	3.2	3.88	1.2
1.00	..	..	..	..	..	..	..	..	..	..	..	..	0.32	3.2	3.83	1.3
1.25	..	..	..	..	..	..	..	17.4	..	..	..	..	0.28	3.1	4.07	1.1
3.00	..	..	..	..	0.22	0.34	0.58	17.4	3.07	..	..	..	..	..	..	..
5.00	0.30	1.50	0.36	0.60	0.26	0.28	0.52	16.8	2.92	0.25	0.39	0.34	..	..	..	..
7.00	..	..	..	..	0.23	0.36	0.52	17.3	2.86	0.24	0.43	0.35	..	..	..	..
10.00	0.29	1.46	0.27	0.52	0.24	0.35	0.53	..	2.83	0.27	0.40	0.36	..	..	..	..
15.00	0.29	1.45	0.29	0.63	..	..	..	..	3.08	0.25	0.38	0.34	..	..	..	..
20.00	0.31	1.45	0.29	0.58	..	..	..	..	..	..	..	..	..	..	..	..
Av.	0.30	1.47	0.30	0.58	0.24	0.33	0.54	17.2	2.95	0.25	0.40	0.35	0.30	3.2	3.93	1.2

[a] All measurements made using same 0.128-mm. cell; to simplify calculations all absorptivities referred to 0.128-mm. thickness.

A_{solvent} = absorbance of cyclohexane at the particular wave length. Calculated by multiplying the absorptivity as listed in Table III by the per cent of cyclohexane in solution—i.e., 10% solution of 2,4-dinitrotoluene corresponds to 90% cyclohexane

The absorptivities of the ingredients at various concentrations at the four designated points are listed in Table IV (constant slit program and same 0.128-mm. cell were used). The small differences in the absorptivity values obtained for each component at different concentrations appeared random in nature and were attributed to experimental error and to error inherent in the instrument. Therefore the values were averaged for each component over the entire range of concentration of each wave length, and the resultant average absorptivities were used in the subsequent analysis of the synthetic samples.

Determination of Ingredients. In general, a 20% solution of the sample in cyclohexane was prepared for the analysis. However, in some cases, a more or less dilute solution was also desirable, depending on the amounts of each ingredient present in the sample for which the absorbance was measured. Optimum absorbance measurements (*3*) are obtained at 37% transmittance (absorbance = 0.43). Therefore, if when using the 20% solution any measurement fell outside of 20 to 65% transmittance, an additional solution was made, stronger or weaker than 20%, so that the reading would fall within 20 to 65% transmittance. Using the 0.128-mm. cell the absorbance of the solution was measured at the peaks of the 8.49-, 8.70-, 9.39-, and 12.49-micron bands. The percentages of the components were calculated by the method of successive approximations (*1*), making the calculations for each component in the following sequence: *o*-, *p*-, *m*-nitrotoluene and 2,4-dinitrotoluene (expected order of decreasing concentration of the ingredients in actual samples).

The first approximation of the *o*-nitrotoluene was obtained as follows:

$$\%\ o\text{-nitrotoluene} = \frac{(A_{\text{total}} - A_{\text{solvent}})\ 100\ s}{a \times W}$$

where

A_{total} = absorbance of cell filled with the cyclohexane solution at 8.70 microns
A_{cell} = absorbance of cell at 8.70 microns
A_{solvent} = absorbance of cyclohexane at 8.70 microns. (This value is obtained by multiplying the absorptivity of cyclohexane (Table III) at 8.70 microns by the percentage of cyclohexane in solution—i.e., a 20% solution of the sample contains 80% cyclohexane
s = weight of cyclohexane solution in grams
W = weight of sample used in making the solution in grams
a = absorptivity of *o*-nitrotoluene at 8.70 microns (average value from Table IV)

Although the bands to be measured in the determinaton of each component were selected so as to keep interference due to the other constituents at a minimum, there is some absorbance at each band that is due to other than the component for which the measurement is made. This interfering absorbance must be corrected for. However, since no previous knowledge was available as to the amount of each constituent prior to the first approximation of the *o*-nitrotoluene, no accurate correction could be applied for the ingredients (*m*-, *p*-nitrotoluene and 2,4-dinitrotoluene). Therefore, they were disregarded and as a consequence the value obtained for *o*-nitrotoluene was only approximate (first approximation).

The first approximation of *p*-nitrotoluene was obtained as follows:

$$\%\ p\text{-nitrotoluene} = \frac{(A_{\text{total}} - A_{\text{cell}} - A_{\text{solvent}} - A_{o\text{-MNT}})\ 100\ s}{a \times W}$$

where

A_{total} = absorbance of cell filled with cyclohexane solution at 8.49 microns
A_{cell} = absorbance of cell at 8.49 microns
A_{solvent} = absorbance of cyclohexane at 8.49 microns
$A_{o\text{-MNT}}$ = absorbance at 8.49 microns due to the *o*-nitrotoluene obtained as shown in the following example: Assuming that the percentage of *o*-nitrotoluene found in the previous approximation was 50% and the sample was a 20% cyclohexane solution, then

$$A_{o\text{-MNT}} = \frac{50}{100} \times \frac{20}{100} \times 0.30 \text{ (Table IV)} = 0.030$$

s = weight of cyclohexane solution in grams
W = weight of sample used to make the cyclohexane solution in grams
a = absorptivity of *p*-nitrotoluene at 8.49 microns

[The value obtained is only approximate (first approximation) because the contribution of *m*-nitrotoluene and 2,4-dinitrotoluene have been disregarded and only an approximate correction has been made for the contribution by the *o*-nitrotoluene.]

The first approximation of *m*-nitrotoluene was obtained in a manner similar to that used for the *o*- and *p*-nitrotoluene, except that now the approximate amounts of *o*- and *p*-nitrotoluene were known and a correction for each was applied.

The first approximation of 2,4-dinitrotoluene was calculated in a manner similar to that used for *o*-, *m*-, and *p*-nitrotoluene, except that the approximate corrections for each of these was applied.

Having obtained the first approximations for all the components, the whole procedure was repeated to obtain the second approximations. In the second approximation of *o*-nitrotoluene, the corrections for *p*- and *m*-nitrotoluene and 2,4-dinitrotoluene were obtained by using the values for each of these obtained in the first approximations. In a similar manner the second approximations for *p*- and *m*-nitrotoluene and 2,4-dinitrotoluene were obtained, each time using the best (latest approximations) calculated values of the other components to obtain correction values to be applied to the component being calculated.

The process described above was repeated until the values obtained in a subsequent approximation were the same as those of the previous approximation. It has been found by experience that in the analysis of unknown mixtures about three approximations were generally sufficient to achieve the final values.

DISCUSSION OF METHOD AND RESULTS

The synthetic samples used to establish the developed method covered wide ranges of concentration for each ingredient. *o*- and *p*-nitrotoluene were present in greater concentrations than *m*-nitrotoluene and 2,4-dinitrotoluene because that is what is expected to be encountered in actual samples of nitrations of toluene. Although the results listed in Table II show the method to be applicable over wide ranges of concentration for each ingredient, there appears to be no reason why the method should not be applicable over the complete range of 0 to 100% except in the case of 2,4-dinitrotoluene which has a relatively large correction at 8.70 microns where *o*-nitrotoluene is measured. (The concentration of 2,4-dinitrotoluene in actual samples, however, is expected to be very low.)

The results listed in Table I show that, when an ingredient is present in amounts greater than 2 or 3%, the relative error and the coefficient of variation are rather small considering the nature of the method which involves the analysis of a four component mixture of isomers and related compounds. When the ingredients are 2% or less, as expected, the relative error and the coefficient of variation are in some cases rather high. This is caused by the fact that the relative error and the coefficient of variation are adversely affected by the small concentration. However, even in these cases, as judged by the absolute error and the standard deviation, the results obtained using the developed method are very useful.

In spectrophotometric measurements maximum precision is obtained at 37% transmittance (*3*); however, the precision does not suffer very much unless the measurement is made at much greater or smaller values. For the purpose of obtaining good precision the developed method prescribes the preparation, if necessary, of more than one sample solution so that the absorbance measurement will be made near the optimum point. In general, it was found that two separate solutions of the sample were sufficient to obtain reasonably good measurements.

It has been known (*3*) and confirmed (*1*) that absorptivities vary with slit width. This fact makes it imperative that the slit width used in the analysis of samples be exactly the same as the one used in obtaining the absorptivity constants using standards.

The analysis of the mixture of *o*-, *m*-, and *p*-nitrotoluene and 2,4-dinitrotoluene involves the making of absorbance measurements at four wave lengths corresponding to the four selected infrared bands (one for each ingredient). The four absorption bands were chosen taking into consideration reasonably strong absorbance and freedom from interference from absorption bands of other components. The four selected bands are not completely ideal, as at 8.70 microns (used for the *o*-nitrotoluene) both *o*-nitrotoluene and 2,4-dinitrotoluene have bands whose peaks are at exactly the same wave length. However, because 2,4-dinitrotoluene is usually present in very small amounts in actual samples and also because the two bands are at exactly the same wave length the use of the 8.70-micron band has proved to be satisfactory. Cyclohexane was selected as the solvent because of its relatively small absorbance at the four points of measurement and also because the four components were reasonably soluble in this solvent (2,4-dinitrotoluene is not too soluble in cyclohexane, but in the presence of *o*-, *m*-, and *p*-nitrotoluene, such as present in actual samples, its solubility is adequate for the analysis).

In the process of obtaining absorbance measurements it is sometimes difficult to be certain that the measurement is being made exactly at the point where the maximum absorbance of the particular band occurs. This is especially true when the cell and solvent absorbances are being determined or when the absorbance of the sample is such as to produce a shoulder alongside of another major band instead of producing a well-defined band. This difficulty was surmounted by first locating the position of the true peak, using a reference cell containing a solution of about 5% of each of the ingredients in cyclohexane.

ACKNOWLEDGMENT

The authors wish to express their appreciation to A. J. Clear, C. J. Bain, R. Frye, and J. C. Armitage of Picatinny Arsenal for help rendered in the publication of this report. Appreciation is further expressed to the Ordnance Corps for permission to publish this paper.

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Received for review April 13, 1954. Accepted October 21, 1954.

Application of the Logarithmic Sector to Quantitative Spectrographic Analysis of Petroleum Ash Residues

E. B. CHILDS and J. A. KANEHANN
Technical Service Department, Socony-Vacuum Oil Co., Inc., 412 Greenpoint Ave., Brooklyn 22, N. Y.

A large number of deposits, sludges, and used oil samples are analyzed by petroleum laboratories to furnish clues of proper or improper operation of engines and machines. This investigation was undertaken to provide a rapid quantitative spectrographic procedure for the analysis of these samples. The logarithmic sector method is fast and obviates the need for an expensive densitometer. Analytical procedures, data, and results on 13 different samples are presented. The average standard deviation is 30%. Working time per sample is about 75 minutes for ashing and quantitative determination of eleven elements.

QUANTITATIVE spectrographic methods, used for analyses of many materials, generally rely on densitometric study of the exposed plate to determine line intensities. Another technique for measuring line intensities involves the use of the logarithmic sector. A logarithmic sector disk is similar to a step sector, except that the periphery is cut as a smooth curve (Figure 1). It can be thought of as a step sector with an infinite number of steps. When the sector is rotated in front of the spectrographic slit, light from the source is interrupted. Figure 1 shows that the amount of light will vary at different locations on the slit. Light interruptions at the outside of the sector will be short. In contrast, the blanking of light near the bottom of the V of the sector will be almost continuous. Hence, tapered lines will be formed on the photographic plate of the spectrograph.

Because of the shape of the rotating sector and the response of the photographic emulsion, the length of each line is a logarithmic measure of intensity and the difference in length of two lines is the logarithm of the ratio of the intensities or $\log I_u - \log I_s$, where I_u is the intensity of the element line and I_s is the intensity of the internal standard line. This intensity ratio is generally independent of conditions of exposure, slit width, development, and plate characteristics (*3*). Line differences—i.e., intensity ratios—are plotted against per cent concentration to give a working curve.

Several factors which must be considered when using a logarithmic sector are (*11*):

The jaws of the slit must be parallel and clean.

The sector should be placed directly in front of and as close to the slit as possible.

The straight edge of the sector must be parallel to the edges of the slit when it revolves past the slit.

The sector should revolve at least 50 to 100 revolutions during each exposure.

Each spectrum line must be uniform along its length when the logarithmic sector is not used.

An image of the source should be formed on the prism (or grating), not on the slit.

The spectrograph must be stigmatic or special arrangements must be made to overcome this difficulty. Most grating instruments are not stigmatic.

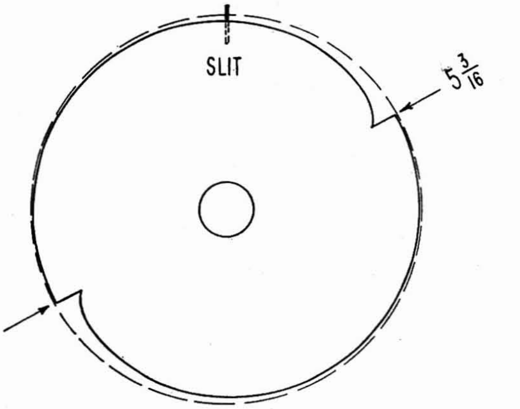

Figure 1. Logarithmic sector disk

Scheibe and Neuhausser (*9*) are credited with first application of the logarithmic sector to quantitative spectrographic analysis in 1928. Twyman and Hitchen (*13*) analyzed dried powders (salts, etc.) by packing directly into crated graphite electrodes. Accuracy of 10 to 20% was claimed. Slavin (*10*) also used this procedure for determination of cobalt, iron, copper, cadmium, thallium, germanium, and lead in metallic zinc and zinc sulfate solutions. Foster and Horton (*4*) used a special optical comparator with a fixed line which is moved along the analysis line until they taper off equally. This method helps define the terminus of the line. Several advantages of the logarithmic sector over the densitometer listed by Green (*5*) include speed, self-calibration, and lack of special equipment. Many others (*1, 2, 6, 7, 11–15*) investigated the application of the logarithmic sector to spectrographic analysis. A complete bibliography of the logarithmic sector applications is given by Rankama and Joensuu (*8*).

In order to apply the logarithmic sector to analyses of various products, an internal standard (indium) was employed. Indium shows a very simple spectrum in the 2500 to 3500 A. region. The internal standard line, 2932.6 A., is almost in the middle of the analytical region; thus, any large variations in plate emulsions are eliminated. The use of a logarithmic sector makes a densitometer and plate calibration unnecessary. However, the other spectrographic techniques remain the same.

EQUIPMENT

A Bausch and Lomb large Littrow spectrograph with the camera positioned to photograph the 2500 to 3500 A. range is used. The plates employed are Eastman Kodak, spectrum analysis No. 1. Exposure time is controlled automatically.

All electrodes are high purity spectrographic grade graphite. The anode is 2 inches long and $^1/_8$ inch in diameter, flat cut. The cathode is 1.5 inches long and $^1/_4$ inch in diameter, with the end a 30° cone containing an axial $^1/_{16}$-inch diameter crater, $^1/_{16}$ inch deep.

A standard 5000 r.p.m. motor with a $5^3/_{16}$-inch logarithmic sector disk (Jarrell Ash Co.) is placed in the optical path.

A direct current motor generator, 115 volts, 60 amperes, is used as a source. The arc is initiated by a radio-frequency–generated spark. Two current levels are used. The 7.5-ampere level is used first to prevent "popping" of the sample. After 5 seconds, the current is increased to maintain sensitivity.

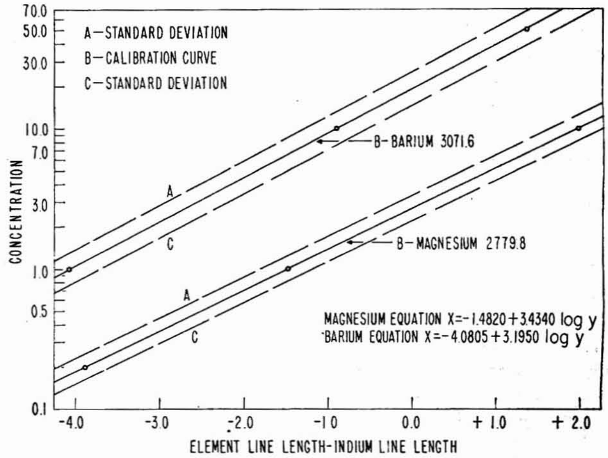

Figure 2. Typical analytical curves and standard deviations

The plates are placed on a special viewing box and read with a modified Bausch and Lomb optical magnifier.

Other auxiliary equipment includes muffle furnaces, analytical balance, grinders, and general analytical laboratory facilities.

METHOD

Standards, Internal Standard, and Buffer. Prepare two stock synthetic ashes by combining pure compounds of the various elements. Stock ash A contains four elements: iron, silicon, lead, and barium. Stock ash B contains eight elements: aluminum, magnesium, tin, chromium, calcium, copper, silver, and sodium. Use oxides and carbonates in preparing these standards, except where these compounds are hygroscopic or deliquescent. To ensure purity, analyze spectrographically all the compounds used in formulating the standards. Make blends of stock ashes A and B and dilute with lithium carbonate to give known concentrations of the various metals. The range of concentrations covered is listed in Table I.

To the final standards add enough lithium carbonate to equal 10 times the quantity of synthetic standard used. Finally add 1% of indium as the oxide to the resultant mixture of buffer and standard. Arc the standard samples and develop the plates as described in the section on analysis of samples.

Table I. Analytical Lines and Concentration Ranges

Element	Wave Length, A.	Element in Ash, %
Aluminum	2660.4	0.3–10
	3092.7	0.02–0.5
Magnesium	2779.8	0.2–10
	2795.5	0.01–0.2
	2833.1	0.2–50
Lead	2833.1	0.2–50
Tin	2840.0	0.02–1.0
Silicon	2881.6	0.1–50
Iron	2983.5	0.2–50
Chromium	3014.5	0.02–1.0
Barium	3071.6	1–50
Calcium	3158.9	0.5–10
	3179.3	0.5–10
Copper	3247.5	0.04–1.0
	3274.0	0.04–1.0
Silver	3280.7	0.01–1.0
Indium (intern. std.)	2932.6	.....

Table II. Arcing and Exposure Conditions[a]

Type of excitation	D.c. arc with radio frequency initiating spark
Circuit constants	
Voltage across electrodes	45–55
Current	5 sec. at 7.5 amp., 20 sec. at 16 amp.
Capacitance, mf.	0.4
Resistance, ohms	20
Arc gap, mm.	5
Spectral region, A.	2500–3500
Slit width, microns	20
Height, mm.	13
Exposure time, sec.	25 (5 at 7.5 amp., 20 at 16 amp.)
Log sector disk and motor	

[a] No condensing lenses, diaphragms, or filters are used.

Draw calibration curves by plotting the difference in line lengths of the element line and internal standard line against the concentration (log scale) on semilog paper.

Table I gives the element lines used. Two typical calibration curves (with standard deviation) are shown in Figure 2.

Analysis of Samples. Thoroughly mix the sample in its original container by vigorous shaking. Viscous samples require heating in addition to shaking. Dry samples must be ground in a mortar. Samples not requiring ash treatment are run directly after mixing.

Weigh to the nearest 0.01 gram a sufficient sample to yield about 0.05 gram of ash residue. Ignite the sample in a porcelain crucible and burn gently to a carbon ash. Cool and add one drop of concentrated sulfuric acid. Heat slowly to drive off sulfuric acid fumes and put the sample into a muffle furnace maintained at 1000° ± 50° F. until all the carbon is consumed. Cool in a desiccator and weigh the ash to the nearest 0.0001 gram.

Thoroughly mix the ash and weigh 0.01 gram into a screw-cap vial. Add 10 times as much lithium carbonate to the vial and to the final mixture add 1% indium as the oxide. Grind the mixture in a mortar and store in the original vial.

Pack the mixture into a cratered electrode by pushing the hollow end of the electrode into some of the sample contained in the cap of the vial. Set the spectrograph as indicated in Table II and arc the sample.

Develop the Eastman Kodak spectrum analysis No. 1 plates for 2.5 minutes at 68° F. in EK-D19 developer with constant agitation. Short stop by a 10-second immersion in 3% acetic acid. Fix for 5 minutes in EK rapid liquid fixer with hardener. Wash for 10 minutes in circulating cold water and dry in a drying oven.

The ratio of intensities between two lines is measured by their difference in lengths. The length of the line may be measured from any spot that is common to all lines. In the method used here a hairline was fixed permanently to the 20-micron slit. This hairline served as a common base for the measurements. Measurements are performed with a modified Bausch and Lomb magnifier. The modification consists of a small piece of emulsion containing a typical logarithmic line attached to the face of the magnifier (Figure 3). With the logarithmic line on the eyepiece direct intensity comparison is facilitated. Determine the length of the line by moving the eyepiece along the line being measured until the rate of extinction of the lines is the same. Estimate the line length to the nearest 0.05 mm., using the scale in the magnifier.

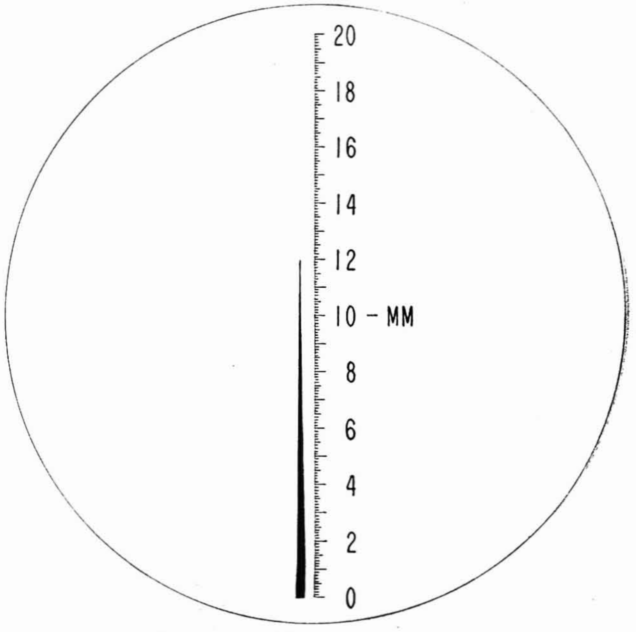

Figure 3. Measuring eyepiece

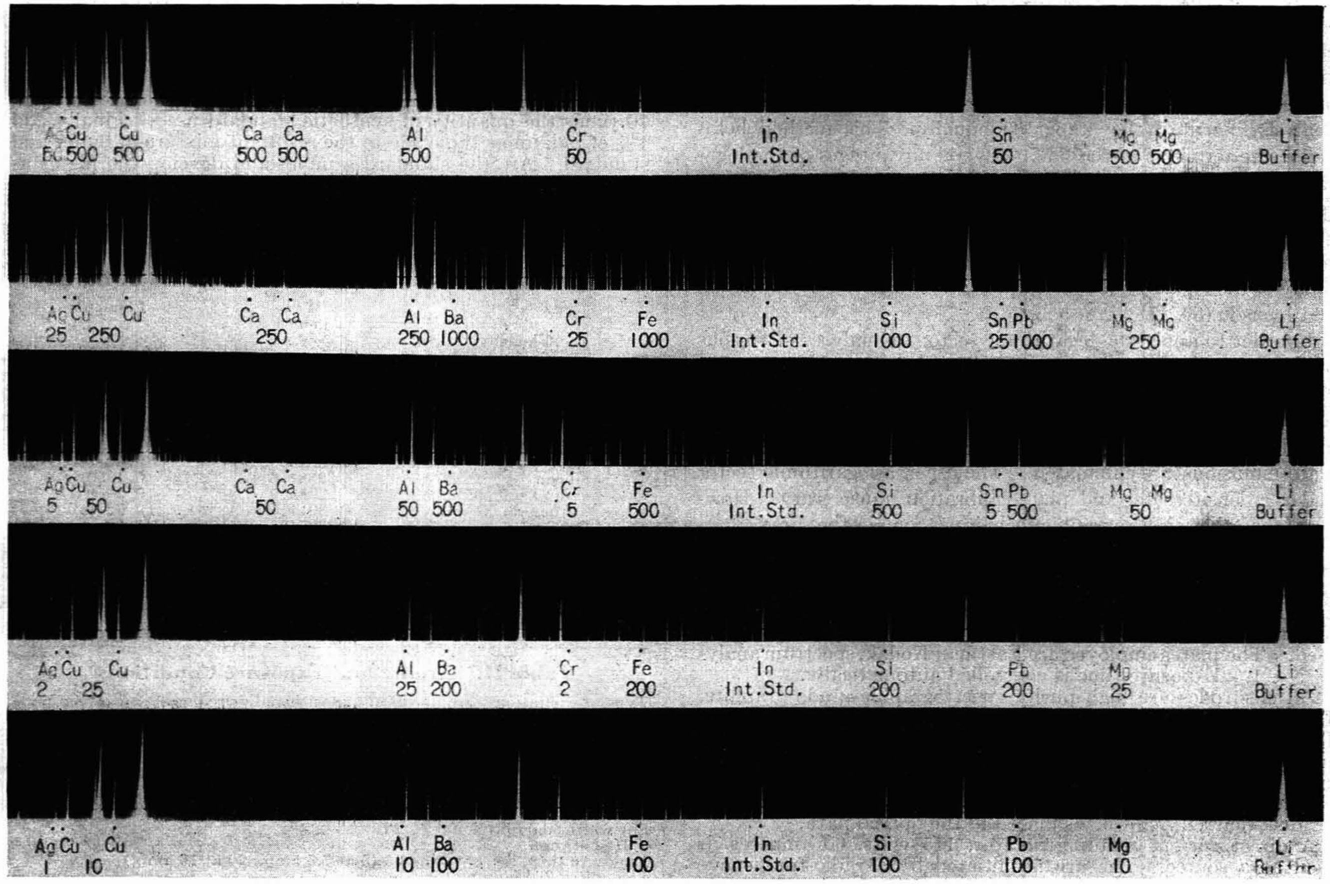

Figure 4. Typical plate

Line lengths proportional to concentration

Figure 4 indicates how the length of the various lines decreases with decreasing concentration. The use of a constant amount of an internal standard in conjunction with the logarithmic sector makes plate calibration unnecessary.

DISCUSSION

This method can be applied to any powder or sample of ash residue. It has been successfully applied to many types of samples, such as used engine oils, greases, cracking catalysts, and deposits. The analyses of 13 samples are shown in Table III. The five cracking catalyst samples were supplied by the Subcommittee on Analysis of Cracking Catalysts, Committee on Analytical Research of the American Petroleum Institute. One sample (used oil B) was supplied by the Pennsylvania Railroad laboratories as a member of the ASTM Committee D-2, Research Division III, Section E on Spectrographic Analysis of Lubricating Oils. Results obtained by chemical analyses are shown also. These samples were used only as reference samples to show the universal application of the method.

The method is a valuable adjunct to the usual major-minor trace analysis and the longer, more complicated densitometric procedure. It is particularly suitable for samples requiring somewhat less precise results than given by chemical analysis. In this laboratory operator time is about 1.25 hours, in contrast to about 2 hours for the densitometric method or 24 hours for chemical analysis of the 11 elements.

ACCURACY AND PRECISION

The correlation between standardization data and known elemental concentration is very high (average correlation coefficient for all elements is 0.96).

In Table III results are presented on a variety of samples. The accuracy of the method was calculated from the National Bureau of Standards samples and equals ± 22.8%. However, in two cases—namely, aluminum in NBS 88 and chromium in NBS 97—the errors are very large (72 and 80%, respectively). These errors are noticed in the 0.05% concentration range, which equals about 2.5 p.p.m. in the original oil sample. The accuracy for the National Bureau Standards, disregarding these two questionable results, is within ±12.2%.

The standard deviation of the mean for all samples is ±30%. In the standard deviation calculations the chemical results were taken as the true value.

Table III. Experimental Results

Sample	Ash, %	Logarithmic Sector, P.P.M. 1	2	3	Av.	Chemical, P.P.M.
Grease ash	0.75					
Copper		0.8	0.9	1.0	0.9	<0.8
Magnesium		4.5	1.1	1.5	2.3	....
Used oil (Diesel) ash A	0.56					
Aluminum		5.3	7.3	7.9	6.8	....
Magnesium		1.2	1.8	1.8	1.6	<0.6
Silicon		5.0	5.0	7.3	5.6	5.1
Iron		31	26	44	33	20.8
Barium		2520	2580	2900	2660	2028
Copper		1.7	1.7	1.7	1.7	0.1
Used oil (ash residue) B	0.46					
Aluminum		5.5	5.6	3.5	4.9	6.6
Magnesium		3.7	4.1	2.8	3.6	4.1
Lead		110	105	97	104	31
Tin		4.1	3.2	7.3	4.8	2.6
Silicon		16	20	12	16	2.1
Iron		110	105	68	94	47
Barium		3200	3040	...	3100	....
Copper		15.6	13.8	20.0	16.4	22.9
Calcium		194	250	212	237	....
Grease ash	2.46	346	184			
Aluminum		5.9	9.0	...	7.5	8.6
Magnesium		14	34	42	30	24.6
Lead		....	...	...	Too high	21,000
Iron		....	...	...	Too low	4.9
		Logarithmic Sector, % 1	2	3	Av.	Chemical, %
Synthetic catalyst, new						
Aluminum		3.1	3.1	3.2	3.1	6.99
Silicon		30[a]	...	...	...	32.6
Copper		0.01	0.01	0.01	0.01	0.008
Synthetic catalyst, contaminated						
Aluminum		6.0	7.4	...	6.7	5.8
Silicon		>30	>30	...	>30	37.9
Iron		0.4	0.2	0.3	0.3	0.4
Copper		0.01	0.01	0.01	0.01	0.008
Natural catalyst, new						
Aluminum		7.4	7.8	4.8	6.7	7.5
Silicon		>30	>30	>30	>30	29.8
Iron		1.6	1.2	0.5	1.1	1.96
Copper		0.01	0.01	0.01	0.01	0.014
Natural catalyst, contaminated						
Aluminum		3.7	7.4	4.1	5.4	5.5
Silicon		>30	>30	>30	>30	30
Iron		2.5	1.7	1.8	2.0	5.8
Copper		0.03	0.04	0.04	0.04	0.02
NBS 1A (argillaceous limestone)						
Aluminum		0.7	2.4	1.2	1.4	2.2
Magnesium		1.3	1.7	1.6	1.5	1.3
Silicon		4.8	11.0	5.6	7.1	6.6
Iron		0.5	0.8	2.0	1.1	1.14
NBS 88 (dolomite)						
Aluminum		0.07	0.1	0.03	0.06	0.035
NBS 98 (plastic clay)						
Magnesium		0.36	0.36	0.52	0.41	0.43
Iron		1.8	1.8	1.9	1.8	1.4
Copper		<0.01	<0.01	<0.01	<0.01	0.006
NBS 97 (flint clay)						
Magnesium		0.07	0.28	0.15	0.16	0.156
Silicon		23	19	22	21.3	20.2
Iron		0.55	0.72	0.62	0.63	0.69
Chromium		0.072	0.07	0.13	0.09	0.05
Natural synthetic catalyst mix						
Aluminum		7.0	7.0	5.3	6.4	4.8
Iron		0.56	0.4	0.34	0.43	0.72
Silicon		>30	>30	>30	>30	31.1
Copper		<0.01	<0.01	<0.01	<0.01	0.002

[a] Maximum calibration value.

ACKNOWLEDGMENT

The authors gratefully acknowledge the help of Ralph Wareham and Owen Black in statistical analysis of results.

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RECEIVED for review May 26, 1954. Accepted October 20, 1954.

Ultraviolet Spectrophotometric Determination of Polymerized Styrene in Styrenated Fatty Acids and Alkyd Resins

R. C. HIRT, R. W. STAFFORD, F. T. KING[1], and R. G. SCHMITT

Stamford Research Laboratories, American Cyanamid Co., Stamford, Conn.

A rapid method for the determination of polymerized styrene in styrenated fatty acids and alkyd resins has been made desirable by the increased use of styrene for modifying alkyd resins. An ultraviolet spectrophotometric method has been developed, which makes corrections for the overlapping absorption of conjugated triene fatty acids and residual monomeric styrene. The method as tested on a variety of styrenated fatty acids is superior in both speed and accuracy to the chemical method based on saponification value.

THE increased use of styrene for modifying alkyd resins has made desirable a rapid method for determining the polymerized styrene content of either the styrenated fatty acids used in manufacture or the filtrate residue from the Kappelmeier separation of the alkyd resin (*1*). Prior to 1953 the principal approach was that proposed by Kappelmeier (*6*), which was based on saponification value ratios. Since ultraviolet spectrophotometry had been successfully applied to such related problems as the determination of phthalic acid (*9*) in the precipitate from the modified Kappelmeier procedure (*1*), the determination of conjugated polyene acids in fatty acids (*2*, *3*), and the determination of monomeric styrene in polystyrene (*7*, *8*), it seemed plausible that a spectrophotometric procedure might be developed, based on the strong absorption of polystyrene near 269 mμ, if suitable corrections could be applied for other absorbing materials present. Since the completion of this spectrophotometric method, a noninstrumental method has been described by Swann (*11*). These methods have recently been reviewed (*10*).

BASIS OF METHOD

Spectroscopically, polystyrene may be regarded as a monosubstituted benzene. In either the homopolymer (polystyrene) or in a copolymer the chromophoric grouping is the benzene ring attached to the polymer chain, and the absorption wave length is essentially unaffected by the details of the composition of the polymer chain. The absorption intensity at 269 mμ thus gives a measure of the number of benzene rings present regardless of the exact composition of the polymer chain. The term "polymerized styrene" is here used to cover both true homopolymer and any copolymers or interaction products present.

In styrenated fatty acids or oils and in the Kappelmeier filtrate residue, the possible interfering materials are the unreacted monomeric styrene and the conjugated polyene fatty acids or oils. The spectra of polystyrene, monomeric styrene, and conjugated diene and triene acids in cyclohexane solution are shown together in Figure 1. In the 260- to 300-mμ region the absorption of the conjugated diene fatty acid is so low that its contribution may be ignored, even though it may be present in appreciable concentrations. The amounts of conjugated tetraene acids that are present in commercial fatty acids (*2*, *3*) and oils are so low that their contribution is negligible. Thus only conjugated triene acids and monomeric styrene need be considered as interfering materials. Although these both have considerably higher absorptivity than polystyrene, their concentrations are relatively very much lower, and consequently their net contribution to the observed absorbance is smaller but appreciable.

Even though only a one-component analysis with corrections for two other absorbers is actually desired, the overlapping of the absorption requires essentially a three-component analysis with nine known absorptivities.

The longest wave-length maxima of monomeric styrene, conjugated triene acids, and polystyrene occur at 291, 282, and 269 mμ, respectively, so these wave lengths seemed logical choices for the analytical wave lengths. Purified styrene [99.75% pure by the freezing point depression method of Witschonke (*12*)] and polystyrene [reprecipitated, assaying less than 0.14% by the spectrophotometric method of McGovern and coworkers (*7*)] were used to determine the absorptivities for these two compounds. These values checked well with those obtainable from the papers by Newell (*8*) and by McGovern and coworkers (*7*). For the conjugated triene acid, absorptivities were taken from the work of Brice and Swain (*3*, *4*), these being values determined on samples of much higher purity than ordinarily available. The absorptivities of the reference compounds at the analytical wave lengths are shown in Table I.

Table I. Absorptivities of Reference Compounds

Analytical wave lengths, mμ	269	282	291
Polystyrene	1.61	0.0342	0.0175
Conjugated triene acid	214	166.9	8.50
Monomeric styrene	8.27	7.30	5.64

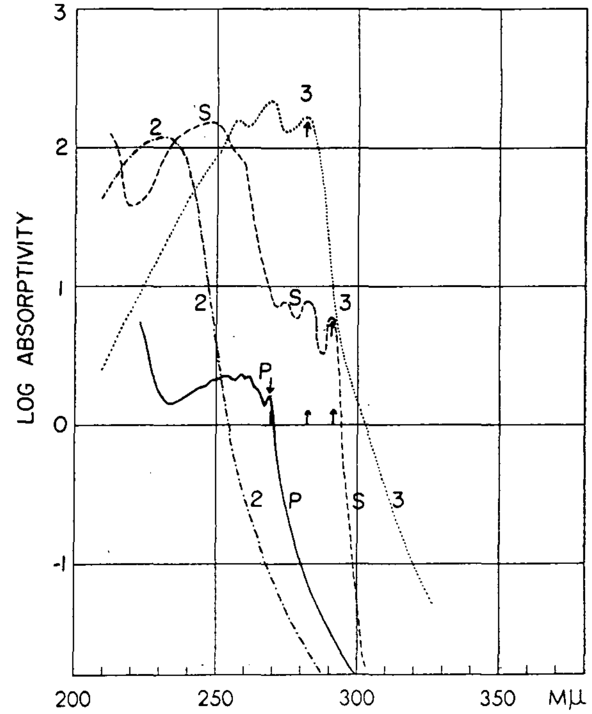

Figure 1. Ultraviolet spectra

Monomeric styrene (*S*, - - - - - - -)
Polymeric styrene (*P*, ———)
Conjugated diene acid (2, ·—·—·—·—)
Conjugated triene acid (3, ...)

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

Table II. Polymerized Styrene Determinations

			Per Cent Polystyrene		
Fatty Acid	Sapon. Value	By Preparation	By ultraviolet spectrophotometry	By Kappelmeier (actual sapon. value)	By Kappelmeier (190 sapon. value)
Saturated	289.7	0.0	0.05	0.0	−52.
		9.5	10.7	12.3	−33.
		30.4	30.6	30.8	−5.0
		48.3	48.0	51.5	26.0
Soybean	201.4	0.0	−1.8	0.0	−6.0
		8.9	7.6	7.7	2.2
		28.4	27.6	27.0	22.6
		49.0	48.6	44.3	41.0
Castor	200.5	0.0	1.6	0.0	−5.0
		8.9	9.3	8.0	3.0
		27.8	29.4	28.9	25.0
		48.5	47.8	48.6	45.7
Linseed	206.5	0.0	1.0	0.0	−9.0
		5.6	7.3	3.8	−4.0
		25.0	25.1	23.4	16.8
		46.7	46.8	. . .	. . .

Using Beer's law in its general form, at a given wave length,

$$A^{\text{obsd.}} = b \sum_i a_i c_i \tag{1}$$

where A is the observed absorbance at the stated wave length, b is the cell length in millimeters, a_i is the absorptivity of component i, and c_i is the concentration (in grams per 100 ml.) of component i. There also exists the relation that $c_i = c_s \times C_i$ where c_s is the concentration of the sample in solution (in grams per 100 ml.) and C_i is the relative concentration, or fraction, of component i. The nine absorptivity values of Table I may be fitted into three simultaneous equations for the observed absorbances at the three selected analytical wave lengths where the

$$A_{291}^{\text{obsd.}} = c_s b\,(0.0175\ C_p + 8.50\ C_t + 5.64\ C_m) \tag{2}$$

$$A_{282}^{\text{obsd.}} = c_s b\,(0.0342\ C_p + 166.9\ C_t + 7.30\ C_m) \tag{3}$$

$$A_{269}^{\text{obsd.}} = c_s b\,(1.61\ C_p + 214\ C_t + 8.27\ C_m) \tag{4}$$

subscripts p, t, and m represent polymerized styrene, conjugated triene acids, and monomeric styrene, respectively.

As it is the concentration of polymerized styrene in the original sample that is desired, and this is the same as C_p, above, the equations may be solved for C_p, and the numerical terms rearranged and combined, giving

$$C_p = \frac{0.6373}{c_s b}\,(A_{269}^{\text{obsd.}} - 1.293\ A_{282}^{\text{obsd.}} + 0.207\ A_{291}^{\text{obsd.}}) \tag{5}$$

This equation may be applied to data in this form, unless the analyst desires to re-evaluate some or all of the absorptivities given in Table I on his available instruments and with samples of his own choosing.

It may be seen in application that Equation 5 acts as a one-component analytical equation with one negative and one positive correction term, and that A_{269} contributes the largest amount, with the $1.293A_{282}$ term considerably smaller correction, and the $0.207A_{291}$ term even less. Fortunately, the two correction terms virtually cancel out a constant or slight rising (toward shorter wave lengths) background.

If the A_{291} reading becomes appreciable in respect to the A_{269} and A_{282} readings, it generally signifies an unusually high concentration of residual monomeric styrene, and Equations 2, 3, and 4 may be solved to permit an estimate of the monomeric styrene concentration to be made. This equation is

$$C_m = 0.00967\left[\frac{1}{c_s b}\,(19.64\ A_{291}^{\text{obsd.}} - A_{282}^{\text{obsd.}}) - 0.310\ C_p\right] \tag{6}$$

An estimate of the conjugated triene acid concentration, C_t, may be obtained by substituting values of C_p and C_m, obtained from Equations 5 and 6 into any of Equations 2, 3, or 4, and solving for C_t.

APPARATUS

The data on standards and test samples were obtained by use of a Cary automatic recording spectrophotometer, Model 11, No. 67, using 1-, 10-, 20-, and 50-mm. quartz cells and micrometer Baly cells (*5*) which were adjustable from 5.00 to 0.02 mm. in length. Any spectrophotometer capable of measurements at the analytical wave lengths with reasonably narrow slits may be used, and the sample concentration and cell length can be adjusted to suit the preference of the analyst. Purified cyclohexane was used as the solvent throughout.

TEST SAMPLES

In order to test the spectrophotometric method, specially prepared samples of styrenated fatty acids were made up. Care was taken to obtain good material balance, and escaping vapors were trapped and analyzed spectrophotometrically for monomeric styrene (*7*). Fatty acid samples were selected to give a variety of commercially available acids, with saturated, random diene (soybean), conjugated diene (castor), and random triene (linseed) acids represented. These were made up to contain roughly 10, 30, and 50% of polymerized styrene. Styrene was polymerized in the presence of the fatty acid, using di-*tert*-butyl peroxide as the catalyst, at 170° C. for 3 to 5 hours. At 200° C the mixture was blown with nitrogen and the volatile fraction was collected and analyzed spectrophotometrically for monomeric styrene (*7*); this value was used as a correction to the composition by preparation.

These samples were also analyzed by the method proposed by Kappelmeier (*6*). Here it was assumed that all the acids were C_{18} acids and that the saponification value of the acids alone would be 190. Polystyrene acts as an inert diluent and may be estimated from the ratio of the saponification value of the styrene–fatty acid mixture to that of the fatty acids alone. In addition, to ensure optimum conditions for this approach, the saponification value determined for each acid (instead of an arbitrary 190) was also used to estimate the styrene content. These data are summarized in Table II, where the polymerized styrene content by preparation, by ultraviolet spectrophotometry, and by the corrected and uncorrected Kappelmeier saponification value methods are compared. Data are also included for fatty acid samples which had not had styrene polymerized in their presence.

DISCUSSION

Examination of Table II shows considerably better agreement between the preparational and the ultraviolet data than between the preparational and the corrected Kappelmeier saponification data; the uncorrected Kappelmeier saponification data are comparable only when the saponification values are near 190 and then only at the higher polystyrene concentrations.

Thus the spectrophotometric method may readily be applied as a direct method to samples of unknown qualitative composition for which the saponification value of the original fatty acid component is not known and cannot be obtained.

The effectiveness of the correction term for the conjugated triene acids is shown by the agreement within 2% of the preparations and the ultraviolet spectrophotometric data on the unreacted fatty acids, where the only absorption present is due to the conjugated triene acids (*2*, *3*). However, an attempt to use the ultraviolet spectrophotometric method on styrenated tung oil acids was unsuccessful; the very high concentrations of conjugated triene acids encountered could not be handled by the correction terms of the equation. Although styrenated tung oil acids are not generally considered to be economically important and are unlikely to be encountered, their presence would be at once apparent by the appearance of the spectrum, which would no longer resemble that of polystyrene. A possible means of handling samples with very high conjugated triene acid concentrations may result from the destruction of the conjugated unsaturation by halogenation or hydrogenation of the sample prior to examination.

To test the limit of effectiveness of the correction term for conjugated triene acids, mixtures were made of tung oil acid and styrenated linseed acid, and the samples were analyzed spectrophotometrically. The data are shown in Table III.

It appears that the conjugated triene correction term is effective up to about 6% conjugated triene acids in the presence of about 20% polymerized styrene. The error in the amount of polymerized styrene increases as the differences between the terms of Equation 5 become of the same order as the photometric error and as the contribution of the trace amounts of conjugated tetraene becomes appreciable; the absorptivity of the conjugated tetraene acid (*2*, *3*) is 10^4 greater than that of polystyrene at 291 mμ.

Table III. Effect of Conjugated Acids

Sample Description	Per Cent Conjugated		Per Cent Polystyrene	
	Triene acids	Tetraene acids	By preparation	By ultraviolet spectrophotometry
Linseed	0.12	0.040	25.0	25.1
19:1, linseed-tung	2.3	0.053	24.3	23.3
6:1, linseed-tung	6.2	0.076	22.6	19.3
3:1, linseed-tung	11.8	0.103	20.2	13.7

APPLICATION TO ALKYD RESINS

The details of the application of this spectrophotometric method to the analysis of resins have been described by Stafford and coworkers (*10*) and are summarized here. The ASTM method (*1*) is applied to the alkyd resin as received. The sample is saponified and the dibasic acids are isolated by precipitation as insoluble potassium salts, in which the phthalic acid may be determined by the ultraviolet spectrophotometric Shreve method (*9*). The filtrate is neutralized with concentrated hydrochloric acid, and the precipitated potassium chloride is filtered off. The filtrate is concentrated, transferred to a separatory funnel, and extracted with ether. The ether layer, which contains polystyrene, fatty acids, and interaction products of these, is evaporated to dryness. A sample of this fraction is weighed and dissolved in cyclohexane for spectrophotometric examination.

ACKNOWLEDGMENT

The authors wish to acknowledge the assistance of William G. Deichert with sample preparation and separation and of Mrs. E. S. Davis and John G. Koren with the spectrophotometric work.

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Received for review August 25, 1954. Accepted November 9, 1954. Presented before the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 5, 1954.

Far Ultraviolet Absorption Spectra of Unsaturated and Aromatic Hydrocarbons

L. C. JONES, JR., and L. W. TAYLOR
Wood River Research Laboratory, Shell Oil Co., Wood River, Ill.

A survey of the analytical potentialities of absorption spectroscopy in the far ultraviolet region has been initiated. In the first phase of this program the far ultraviolet (1700 to 2300 A.) spectra of 69 pure hydrocarbons have been measured either in the vapor or solution state with a recording vacuum spectrometer, employing a 1-meter concave grating and a photoelectric detector. Experimental methods are described and spectra of representative paraffins, naphthenes, cyclic and noncyclic mono-olefins, allenes, conjugated and nonconjugated diolefins, acetylenes, benzenes, naphthalenes, and polycyclic catacondensed aromatics are presented. Useful correlations between the spectra and structure of these hydrocarbons are discussed. It is concluded that far ultraviolet absorption spectroscopy is a promising new tool for the analyst.

Absorption spectroscopy in the near ultraviolet (2100 to 4000 A.) is one of the most widely used physical methods of analysis. Its popularity can probably be ascribed to two factors: the large number of organic compounds with characteristic absorption in this region, and the availability of well-designed, reliable, and relatively inexpensive commercial instruments. The value of the method in the analysis of petroleum fractions is enhanced by the existence of extensive compilations of accurately measured and uniformly presented spectra of pure hydrocarbons.

This is in marked contrast to the present status of absorption spectroscopy in the far or vacuum ultraviolet region. Despite a half century of academic research in far ultraviolet spectroscopy, the technique has not, to the authors' knowledge, been applied by the analyst. The lack of adequate instrumentation has undoubtedly been a large factor in delaying such application. Until very recently intensity measurements in this region have depended upon photometry with special photographic emulsions of very poor reproducibility. Consequently, emphasis in the early research was largely directed toward accurate measurement of absorption band positions and upon calculation of molecular energy levels. The intensity data obtained in these studies were not of the degree of accuracy required in analytical applications. Recent advances in photographic photometry in the far ultraviolet have improved this situation considerably, but the fact remains that most of the published spectra are of limited value to the analyst. The qualitative and semiquantitative features of these spectra suggested, however, that the far ultraviolet region had potential utility in the analysis of hydrocarbons. The photographic measurements of Carr and Pickett and their collaborators at Mt. Holyoke College (*1*, *12*, *19*, *35*) on the aliphatic olefins, diolefins, and cyclic unsaturates, and of Platt, Klevens, and others at the University of Chicago on the aromatics (*16*, *17*, *21*, *23*) and other unsaturated hydrocarbons (*14*, *18*, *24*, *25*) are of special interest in this connection.

Measurements of analytical accuracy in the far ultraviolet have been made possible in the past few years by the development of recording photoelectric spectrometers (*4, 10, 11, 13, 15, 34, 37–39*). With such an instrument a study has been undertaken of the analytical possibilities of absorption spectroscopy in the 1700- to 2100-A. region. The lower wave-length limit has been determined both by the transmittance limits of available solvents (*22, 27*) and by the lower limit of the hydrogen continuum. The measurements have been extended to 2300 A. on the long wave-length side to permit comparison with previously available data from near ultraviolet spectrometers. Since the primary interest was in the field of petroleum chemistry the first phase of this program has involved the measurement of the spectra of a large number of unsaturated hydrocarbons. The spectra of 69 representative hydrocarbons are reported in this paper. Although this phase of the program is incomplete the results are believed of sufficient interest to warrant a preliminary report at this time.

EXPERIMENTAL METHODS

Spectrometer. A schematic diagram of the spectrometer is shown in Figure 1. The basic component is a Baird vacuum ultraviolet monochromator. A detailed description of this instrument has been given by Tousey *et al.* (*37*). Briefly, it consists of an evacuated 1-meter concave grating spectrometer. The grating is an original Johns Hopkins ruling with 15,000 lines per inch and a ruled area of $1^1/_2 \times 3^3/_4$ inches. The reciprocal dispersion is 16.9 A. per mm. Wave-length scanning is accomplished by translation of the grating about the circumference of the Rowland circle. The wave-length drive permits the spectrum to be scanned at either 36 or 100 A. per minute over the 1700- to 4100-A. region. The monochromator has a counter which reads very nearly in Angstroms. If an extensive spectral region is to be covered, however, it is necessary to apply small corrections to the counter readings because of the slight nonlinearity of dispersion of the grating. A correction curve was constructed from measurements of the counter readings for the central beam and for near ultraviolet lines of the mercury arc. These data were fitted by the method of least squares to a power series form of the equation for the dispersion of a grating at normal incidence:

$$\lambda = a + bR + cR^3 \tag{1}$$

where λ is wave length, R is the counter reading, and a, b, and c are constants. The calibration equation obtained in this manner has been used to calculate the wave lengths of the Schuman-Runge bands of oxygen and of the 1600-A. series of hydrogen lines. Agreement with published results was always within 1 A., which is the accuracy claimed for the instrument by the manufacturer.

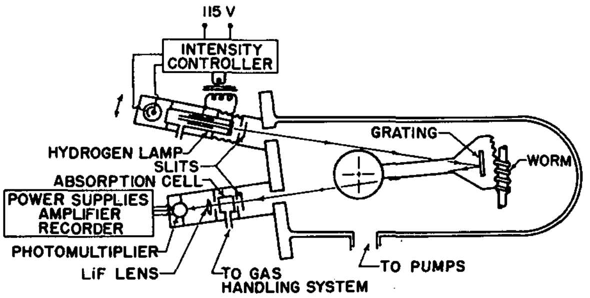

Figure 1. Schematic diagram of far ultraviolet spectrometer

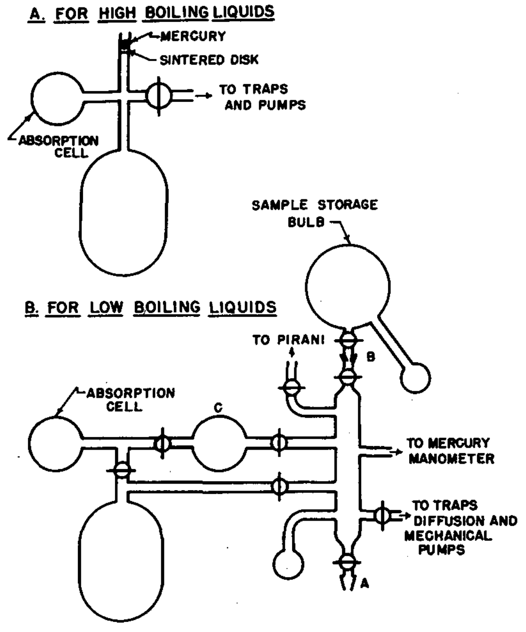

Figure 2. Gas-handling systems

The radiation source is a windowless hydrogen discharge tube, similar in many respects to those described by Powell (*28*) and by Johnson, Watanabe, and Tousey (*15*). However, where they used direct current excitation of the arc, an 1800-volt, 60-cycle power supply was used here to obtain 120 pulses of light per second. This permits use of a stable alternating current amplifier in connection with the photomultiplier detector to obtain improved discrimination against the thermal emission of the multiplier tube and a better signal-to-noise ratio. The lamp is mounted on a bracket which may be rotated about the entrance slit of the spectrometer. This is desirable, since in order to reduce stray light to a minimum the lamp is fitted with a baffle to restrict the cone of illumination entering the spectrometer to that accepted by the grating at any one wave length. In scanning a wide wave-length region the grating will move out of this cone, so that it may be necessary to readjust the position of the lamp. Such adjustment is not necessary if the measurements are restricted to the 1700- to 2300-A. interval as in the present case. The intensity of the lamp is monitored by an ultraviolet-sensitive phototube and maintained constant within about 1% by a servo circuit described by Pondrum and Robertson (*26*).

Located immediately behind the exit slit is the cell compartment which accommodates kinematically mounted absorption cells up to 6 cm. in length for liquids and gases. These are of the lead spacer, amalgam seal type commonly used in infrared spectroscopy, except that they are fitted with windows of synthetic lithium fluoride. Convenient cell lengths for solutions are 0.1 and 0.5 mm. The exact lengths of these cells were determined by measurement of the absorbance at 2500 A. of the cells filled with a solution of diphenyl. An aliquot of the diphenyl solution was then diluted quantitatively so that its absorbance at the same wave length might be measured in the 10.0-mm. Beckman cells. The cell thickness could then be calculated from Beer's law.. All of these measurements were made with a Beckman DU quartz spectrophotometer. It has previously been established that this method gives thicknesses in agreement with the infrared interference fringe method (*36*) within the precision of the latter method. The vapor cell used in the present work is 30.8 mm. in length. It is connected to either of the external gas-handling systems shown in Figure 2 via spherical joints brazed into the cell compartment wall.

A lithium fluoride double convex lens images the exit slit on the photocathode of the photomultiplier. The latter is a special fused-silica envelope 1P28 tube (RCA Development Type C-7139) identical with those described by Dunkelman and Lock (*10*). The multiplier has excellent sensitivity to 1540 A., where it cuts off abruptly. It is operated at a total dynode potential of 840 volts. The output of the multiplier is amplified by a con-

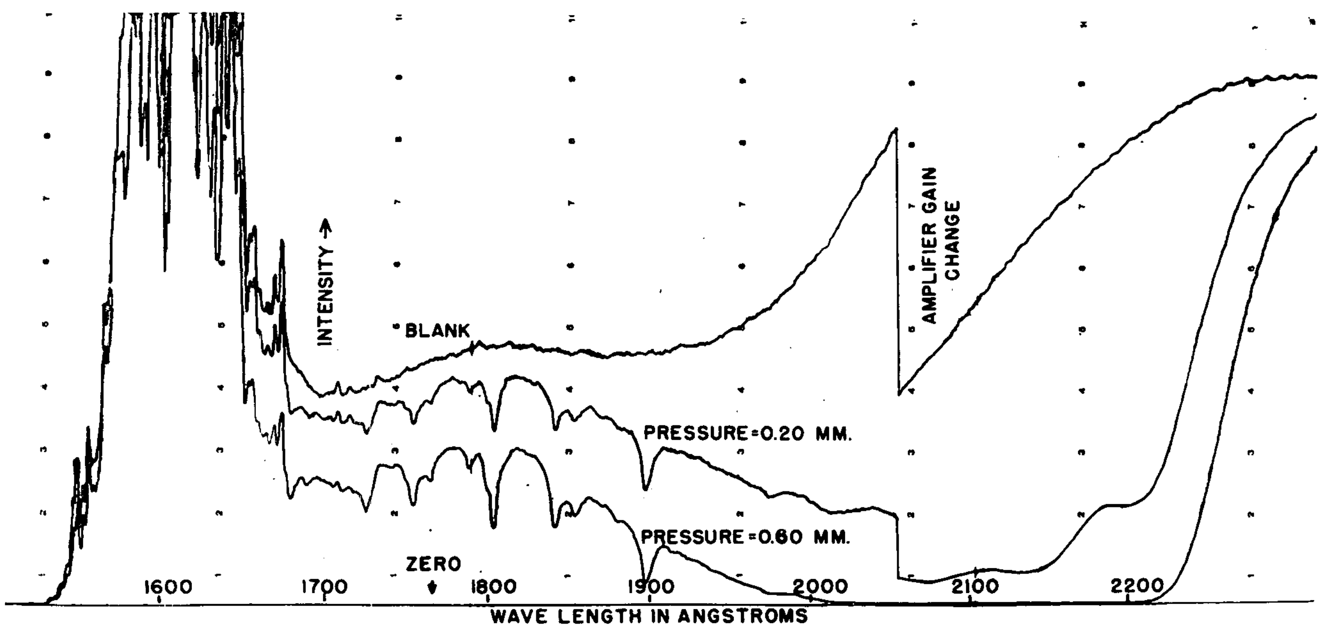

Figure 3. Typical recorded spectrum

ventional, sharply tuned, 120-cycle amplifier supplied by Baird Associates and modified so that the gain might be changed reproducibly and stepwise by factors of $\sqrt{2}$. The output of the amplifier is rectified and fed into a Brown strip chart recorder.

Vapor-Handling Technique. Vapors of condensable compounds are handled in the gas-handling systems of Figure 2. Known amounts of samples which may conveniently be handled as liquids are introduced into the evacuated absorption cell and associated large bulb through a mercury-covered sintered-glass disk (Figure 2,*A*) by means of calibrated micropipets of 5.0- to 0.1-μl. size. The larger pipets were obtained from Microchemical Specialties Co. and the manufacturer's calibration was accepted. The smaller sizes were made especially for the present application and were calibrated gravimetrically with mercury and the calibration was confirmed by means of a mass spectrometer. The more volatile compounds (C_5 and lighter) are handled in the system shown in Figure 2,*B*. The sample is vaporized into the evacuated manifold at *A* or *B* to a pressure of 30 mm. or more. Noncondensable contaminants such as air are removed by freezing the sample in the trap, pumping off uncondensed gases, and re-evaporating. This process is repeated until no residual pressure is detected on freezing out the sample. The sample is then evaporated once more and the pressure measured. A known volume of the vapor is then isolated at this pressure in bulb *C* and the remainder of the system evacuated. The vapor is then expanded from *C* into the large bulb and the absorption cell. If necessary, a second such expansion may be made. In this manner static pressures as low as 0.05 mm. may be obtained with an accuracy of about 1%. The gas-handling system may be heated electrically to facilitate out-gassing. The effect of absorption-desorption phenomena is minimized by carrying out most of the spectral measurements at pressures below 2 mm. of mercury. Satisfactory performance of this method of gas handling has been confirmed by obtaining linear plots of absorbance *vs.* calculated pressures for several olefins at a number of wave lengths. Good agreement between the two methods of gas handling was obtained when both were used with the same sample.

Preparation of Solvent and Solutions. *n*-Heptane is a satisfactory solvent for use in this region of the spectrum (*22*). ASTM knock test reference fuel (*3*), *n*-heptane (Special Products Division, Phillips Petroleum Co., Bartlesville, Okla.), is purified by percolation through Davison 923 grade silica gel. One pound of gel is required per pint of heptane. Solvent prepared in this manner has good transmittance to 1715 A. in 0.1-mm. thickness and to 1740 A. in 0.5-mm. thickness. Solutions were prepared by volumetric dilution of the sample with the solvent. Ultramicropipets and 5.0-ml. volumetric flasks were used for this purpose. Since these pipets are calibrated "to hold" rather than "to deliver" they were carefully rinsed several times with the solvent and the rinsings added to the volumetric flask. Solid samples were weighed into the volumetric flasks with a microbalance.

Operating Technique. All spectra were scanned at 36 A. per minute. A blank energy (I_0) run was made with an evacuated sample cell in the case of the vapor spectra or with a cell filled

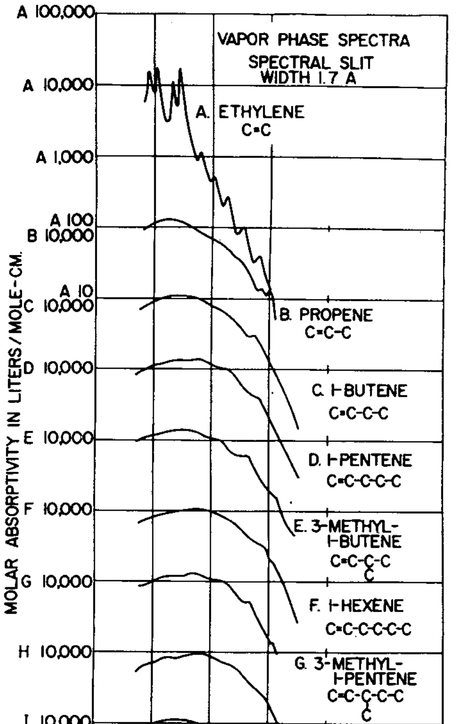

Figure 4. Far ultraviolet spectra of vapors of ethylene and C_3 to C_6 monoalkylethylenes

with the solvent in the case of solutions. The sample vapor or solution was then introduced, the region rescanned, and a second energy (I) trace made. To ensure detection of errors due to unexpected changes in intensity of the lamp, the energy was also measured in a region where the sample was known not to absorb appreciably immediately before each sample run.

A constant spectral band width of 1.7 A. (slit width = 0.1 mm.) has been maintained in all work with vapors. The spectral band width was 5.1 A. (slit width = 0.3 mm.) for solutions. The larger slit width was chosen in the latter case in part because of the poorer transparency of the lithium fluoride windows used in the liquid cell and in part because of the improved signal-to-noise ratio obtained in this manner. None of the solution spectra examined to date has shown any structure which would justify use of narrower slits. A typical vapor spectrum recording is shown in Figure 3. The gain of the amplifier was changed in the middle of the record to maintain a reasonable signal strength at the shorter wave lengths. The abrupt cutoff of the photomultiplier envelope at 1540 A. can be seen. The zero line shown was obtained by scanning with a metal shutter in the light path. The near-coincidence of this line with that of the sample spectrum at 2100 A. and with the blank spectrum below the cutoff is evidence that scattered radiation of unwanted wave lengths is of negligible intensity.

Table I. Source and Purity of Standard Samples

Compound	Source	Purity, Mole %
Ethylene	Phillips[a]	99.96
Propene	Phillips[a]	99.7
1-Butene	Phillips[a]	99.9
cis-2-Butene	Phillips[a]	98.9
trans-2-Butene	Phillips[a]	96.2
2-Methylpropene	Phillips[a]	99.4
1-Pentene	API 281[b]	99.74
cis-2-Pentene	API 282[b]	99.89
trans-2-Pentene	API 283[b]	99.91
2-Methyl-1-butene	API 284[b]	99.86
3-Methyl-1-butene	API 285[b]	99.76
2-Methyl-2-butene	API 286[b]	99.94
1-Hexene	API 519[b]	99.86
cis-2-Hexene	API 526[b]	99.80
trans-2-Hexene	API 527[b]	99.83
cis-3-Hexene	API 528[b]	99.87
trans-3-Hexene	API 529[b]	99.94
2-Methyl-1-pentene	API 530[b]	99.81
3-Methyl-1-pentene	API 531[b]	99.70
4-Methyl-1-pentene	API 532[b]	99.82
2-Methyl-2-pentene	API 533[b]	99.91
3-Methyl-*cis*-2-pentene	API 534[b]	99.85
3-Methyl-*trans*-2-pentene	API 535[b]	99.86
4-Methyl-*cis*-2-pentene	API 537[b]	99.92
4-Methyl-*trans*-2-pentene	API 536[b]	99.75
2-Ethyl-1-butene	API 538[b]	99.90
2,3-Dimethyl-1-butene	API 539[b]	99.86
3,3-Dimethyl-1-butene	API 287[b]	99.91
2,3-Dimethyl-2-butene	API 540[b]	99.90
Cyclopentene	API 288[b]	99.97
Cyclohexene	API 522[b]	99.98
1,3-Butadiene	Shell Oil	99
1-*cis*-3-Pentadiene	API 563[b]	99.92
1-*trans*-3-Pentadiene	API 564[b]	99.92
2-Methyl-1,3-butadiene	API 549[b]	99.96
2,3-Dimethyl-1,3-butadiene	API 570[b]	99.94
1,2-Butadiene	API 512[b]	99.92
1,2-Pentadiene	API 569[b]	99.66
2,3-Pentadiene	API 558[b]	99.85
1,4-Pentadiene	API 565[b]	99.93
1,5-Hexadiene	API 553[b]	99.89
1-Butyne	API 514[b]	99.87
Benzene	API 210[b]	99.98
Toluene	API 211[b]	99.97
o-Xylene	API 213[b]	99.99
m-Xylene	API 214[b]	99.93
p-Xylene	API 215[b]	99.96
1-Methyl-2-ethylbenzene	API 246[b]	99.73
1-Methyl-3-ethylbenzene	API 247[b]	99.57
1-Methyl-4-ethylbenzene	API 248[b]	99.87
1,2,3-Trimethylbenzene	API 249[b]	99.982
1,2,4-Trimethylbenzene	API 250[b]	99.67
1,3,5-Trimethylbenzene	API 251[b]	99.95
1,3-Dimethyl-5-ethylbenzene	API 566[b]	99.89
1,2,3,5-Tetramethylbenzene	API 575[b]	99.92
1,2,4,5-Tetramethylbenzene	API 585[b]	99.86
n-Decylbenzene	API 586[b]	99.80
Tetralin	API 587[b]	99.86
Naphthalene	API 577[b]	99.96
1-Methylnaphthalene	API 578[b]	99.92
2-Methylnaphthalene	API 579[b]	99.91
1,2′-Dinaphthyl	U. S. Bur. Mines[c]	M.p., 76.4–77.4° C.
Phenanthrene	This laboratory	M.p., 100.9–101.2° C.
Anthracene	This laboratory	M.p., 216.0–216.2° C.
Chrysene	U. S. Bur. Mines[c]	M.p., 254–256° C.
1,2-Benzanthracene	Eastman Kodak Co.	M.p., 159–160° C.
20-Methylcholanthrene	Eastman Kodak Co.	M.p., 176–178° C.
2,2,4-Trimethylpentane	API 217[b]	99.96
n-Decylcyclohexane	API 591[b]	99.86

[a] Research grade hydrocarbons from Phillips Petroleum Co.

[b] American Petroleum Institute standard samples of hydrocarbons.

[c] These samples were prepared by M. Orchin of the U. S. Bureau of Mines Laboratory at Bruceton, Pa., and obtained through the courtesy of R. A. Friedel of that laboratory.

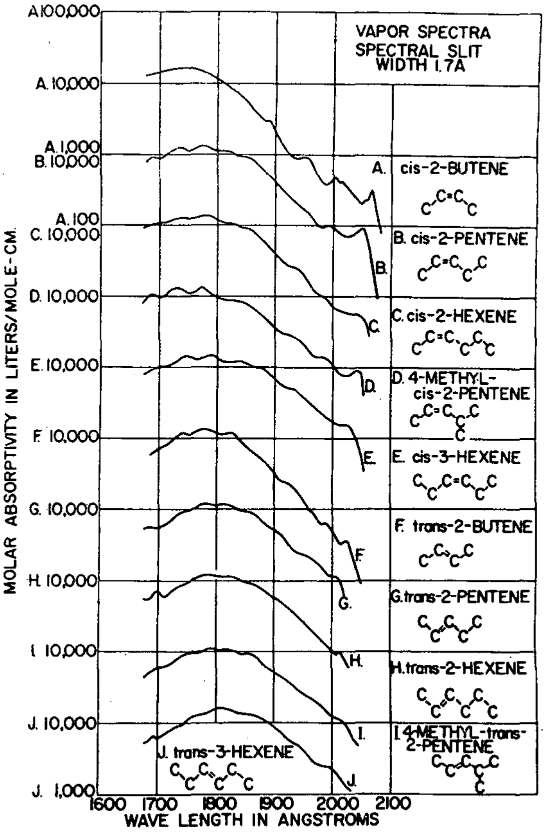

Figure 5. Far ultraviolet spectra of vapors of C_4 to C_6 1,2-dialkylethylenes

Molar absorptivities, ϵ, were calculated from the recorder pen deflections by the usual relationship:

$$\epsilon = (\log_{10} I_0/I)/bc \qquad (2)$$

where b is the cell length in centimeters and c is the concentration in moles per liter. No correction was required for absorption by the solvent, as the solutions were dilute and the transparency of the solvent was high. Due allowance was made for temperature effects and nonideal behavior of the gases in computing the concentrations of the vapor samples. In each case the spectra reported represent the average of three consistent runs, usually made on different days as an additional safeguard against fortuitous repetition of error. Molar absorptivities obtained in this manner normally have a standard deviation of about 3%, so that the 95% confidence limits for the average of the three runs should be approximately ±5% unless some unexpected source of systematic error exists.

Photodecomposition of the sample due to prolonged exposure to high-intensity far ultraviolet radiation was one of the major problems of early workers in this field. This problem has been eliminated completely by the present arrangement of having the absorption cell beyond the exit slit of the spectrometer where it is exposed only to the low-intensity dispersed radiation. The spectra of many of the samples have been scanned repeatedly without finding any detectable change in the absorption spectrum resulting from irradiation.

Standard Samples. The source, and when known, the purity of the compounds used in this work are shown in Table I. Exact data on the purity of some of the samples were not available. In these cases the melting point range as measured with a Kofler micro hot stage (Arthur H. Thomas Co.) and polarizing microscope is given.

COMPARISON OF PRESENT WORK WITH PUBLISHED SPECTRA

Thirty-two of the 69 compounds whose spectra are reported here have been examined by other investigators. The results are compared with those given in the literature in Table II. Of these, Carr and Pickett's data on the C_4, C_5, and C_6 olefins (*1, 12, 35*) and Zelikoff and Watanabe's spectrum of ethylene (*40*) have been reported since completion of the present study.

In general, it has been found that agreement between wave lengths of sharp absorption bands (such as are found in the spectra of ethylene and 1,3-butadiene) found in the present work and those published earlier has been excellent, usually within 1 A. Agreement of band intensities has been somewhat less satisfactory. The only compound of the present series whose spectrum has been measured by photoelectric techniques is ethylene. The present intensity data are systematically about 12% higher than those published earlier although the wave lengths agree within 1 A. in all cases. On the other hand, peak intensities obtained in this work for the C_4 and C_5 olefins and benzene vapor are usually lower than those of the Mt. Holyoke workers (*19*). The cause of this discrepancy is obscure, but may well lie in differences in sample-handling techniques. This is suggested by the fact that agreement seems to be best where the sample pressures were highest (of the order of 0.1 to 3 mm.) and poorest at the lowest pressures. All of the measurements were made at pressures about ten times higher than those of Carr and of Pickett as a result of a tenfold difference in the length of the cells. The absorption bands for many of these compounds are so broad and flat that determination of the wave length of maximum absorption is often subject to considerable uncertainty. Occasional discrepancies of the order of 10 A. are thus not surprising.

No consistent trend can be found in comparing the solution spectra with those of Platt and his colleagues. Differences in intensity are random and average about 15%. This is not inconsistent with the accuracy claimed for his intensity measurements. Again wave lengths of absorption bands agree as well as might be expected with two notable exceptions. Jacobs' and Platt's spectrum for 2-methyl-1,3-butadiene is shifted about 20 A. to shorter wave lengths compared to that of Figure 9, while their spectrum for 1-*cis*-3-pentadiene is shifted about 10 A. in the opposite direction.

Table II. Comparison of Present Work with Published Spectra

Compound	State	Wave Length, A.[a] This work	Wave Length, A.[a] Lit.	Molar Absorptivity This work	Molar Absorptivity Lit.	Molar Absorptivity Spread, %	Ref.
Ethylene	V	1703	1704	17,100	14,800	+14.4	(*40*)
		1730	1730	11,200	10,400	+ 7.4	
		1743	1744	16,800	13,600	+21.0	
		1778	1778	1,120	900	+21.7	
		1802	1802	490	435	+11.9	
		1825	1826	260	230	+12.2	
		1853	1854	99	106	+ 6.7	
		1882	1881	39.5	38	+ 3.8	
					Av.	+12.4	
1-Butene	V	1745	1752	11,200	15,500	−32.2	(*12*)
cis-2-Butene	V	1740	1754	16,000	21,900	−31.1	(*12*)
trans-2-Butene	V	1780	1778	13,000	14,100	− 8.1	(*12*)
2-Methylpropene	V	1882	1883	11,300	14,800	−26.8	(*12*)
1-Pentene	V	1780	1767	13,700	15,350	−12.0	(*1, 35*)
cis-2-Pentene	V	1773	1767	13,500	16,400	−19.4	(*1, 35*)
trans-2-Pentene	V	1812	1802	12,000	13,500	−11.8	(*1, 35*)
2-Methyl-1-butene	V	1883	1879	9,700	11,200	−14.3	(*1, 35*)
3-Methyl-1-butene	V	1753	1761	14,000	13,200	+ 5.9	(*1, 35*)
2-Methyl-2-butene	V	1775	1768	11,000	10,400	+ 5.6	(*1, 35*)
1-Hexene	V	1772	1779	10,800	10,720	+ 0.7	(*12*)
cis-3-Hexene	V	1790	1787	14,700	15,140	− 2.9	(*12*)
2,3-Dimethyl-1-butene	V	1883	1876	9,700	10,960	−12.2	(*12*)
2,3-Dimethyl-2-butene	V	1870	1865	15,600	11,220	+32.6	(*12*)
Cyclopentene	V	1828	1831	13,600	9,340	+37.1	(*19*)
Cyclohexene	V	1755	1754	8,000	8,310	− 3.8	(*19*)
1,3-Butadiene	V	2158	2158.7	...	...	...	(*35*)
		2094	2099.1	...	...	...	
		2035	2035.2	...	...	...	
		1977	1977.8	...	...	...	
		1864	1863.9	...	...	...	
		1754	1754.7	...	...	...	
1-*trans*-3-Pentadiene	V	1803	1807	6,000	5,700	+ 5.1	(*14*)
	V	2148	2138	27,500	25,400	+ 8.0	(*2*)
1-*cis*-3-Pentadiene	V	1895	1883	11,000	7,400	+39.1	(*14*)
	V	2105	2115	19,900	20,100	− 1.0	(*2*)
2-Methyl-1,3-butadiene	V	1910	1890	6,400	6,200	+ 3.2	(*14*)
		1892	1871	7,600	6,000	+23.5	
	V	2155	2155	19,800	21,000	− 5.9	(*2*)
Benzene	V	1760	1759	68,000	76,000	−11.1	(*19*)
					91,000	−29.0	(*32*)
		1764	1766	67,500	77,600	−13.9	(*19*)
					96,000	−35.0	(*32*)
		1791	1790	109,000	126,000	−15.2	(*19*)
					123,000	−13.3	(*32*)
		1804	1803	70,000	69,000	+ 1.4	(*19*)
	S[b]	1840	1835	60,000	47,000	+24.3	(*23*)
		2045	2042	8,000	6,900	+14.8	
Toluene	S	1890	1885	55,000	55,000	0.0	(*23*)
		2047	2050	7,800	8,200	− 5.0	
o-Xylene	S	1918	1905	53,000	59,000	−10.7	
m-Xylene	S	1935	1930	51,000	76,000	−49.0	(*23*)
p-Xylene	S	1920	1925	57,000	62,000	− 8.4	(*21*)
1,3,5-Trimethylbenzene	S	1990	2005	56,000	45,000	+21.4	(*23*)
Naphthalene	S	1840*m*	1840*m*	5,600	5,000	+11.3	(*17*)
		2210	2202	122,000	133,000	− 8.6	
Phenanthrene	S	1875	1872	30,000	31,000	− 3.3	(*17*)
		1970*m*	1965*m*	14,000	12,500	+11.3	
		2115	2122	37,000	33,000	+11.4	
Anthracene	S	1885	1861	21,500	32,000	−39.2	(*17*)
		1975*m*	1960*m*	4,400	5,800	−27.5	
		2205	2208	10,000	14,500	−36.7	
Chrysene	S	1831	1848	49,000	50,000	− 2.0	(*17*)
		1940	1948	18,000	18,500	− 2.7	
		2030*m*	2030*m*	12,400	12,000	+ 3.3	
1,2-Benzanthracene	S	1825	1822	22,500	23,000	− 2.2	(*17*)
		1900*m*	1910*m*	18,000	13,000	+32.3	
		2022	2028	27,000	21,000	+25.0	

[a] Wave lengths correspond to major absorption band peaks, except when followed by *m*, indicating minimum between absorption bands.

[b] Solution in *n*-heptane.

CORRELATIONS BETWEEN FAR ULTRAVIOLET SPECTRA AND MOLECULAR STRUCTURE

The far ultraviolet spectra of related groups of unsaturated hydrocarbons show regularities which should be useful both in qualitative analysis and in quantitative estimation of these compounds in complex mixtures. Some of the more striking correlations will be discussed in detail.

Noncyclic Mono-olefins. The vapor phase spectra of all the aliphatic mono-olefins from ethylene through the hexenes have been measured (Figures 4 to 6). Six of the hexenes have also been examined in *n*-heptane solution (Figure 7). With the exception of ethylene, which is a special case, the olefins have remarkably similar spectra. Each has a broad, intense, and flat absorption band (ϵ about 10,000) with maximum between 1725 and 1940 A. According to current theories this band results from a transition involving one of the nonlocalized unsaturation or π-electrons in molecular orbitals. It is generally designated by the notation $N \rightarrow V$. Overlaying the $N \rightarrow V$ band is a series of moderately intense vibronic

Table III. Average Position and Peak Intensity of Absorption Bands of Vapor Spectra of the Alkenes

Olefin Type	Av. Position of Max., A.	Molar Absorptivity, Liters/Mole Cm.
1-Alkenes	1750 ± 25	11,800 ± 1200
cis-2-Alkenes	1765 ± 15	12,300 ± 200
trans-2-Alkenes	1790 ± 10	11,700 ± 800
2-Alkyl-1-alkenes	1875 ± 15	8,900 ± 1200

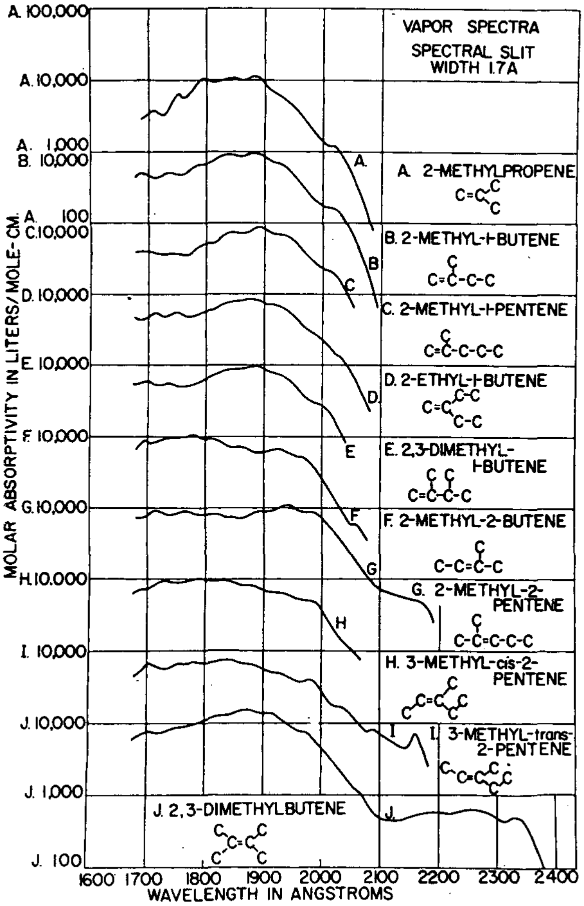

Figure 6. Far ultraviolet spectra of vapors of C_4 to C_6 1,1-dialkylethylenes, trialkylethylenes, and tetramethylethylene

and Rydberg series bands (*5, 29-31*). These are very sharp in the case of ethylene but diffuse for the substituted ethylenes.

For analytical purposes it is convenient to group the olefins according to the number of alkyl groups attached to the carbons of the double bond. Some of these groups must also be subdivided into cis and trans subclasses. Within these groups the far ultraviolet spectra are similar both qualitatively and quantitatively, especially if the lowest member of each series is excluded. These similarities are immediately apparent upon examination of Figures 4 to 6. Within a given class, e.g., 1-alkene or *cis*-2-alkene, all of the spectra lie within an area representing an intensity spread of about 10 to 20% of the mean. The peak intensities fall within an even narrower range as shown in Table III. The spectra of the tri- and tetraalkylethylenes are not included in the correlations of Table III, as an insufficient number of these compounds has been run to establish reliable trends. Thus the spectra of three of the four trialkylethylenes show a strong family resemblance while the fourth, 2-methyl-2-pentene, is anomalous (Figure 6). Only one of the tetraalkylethylenes was included in the present study.

In preparing Table III the average peak position and intensity have been determined from smoothed curves,—i.e., the vibronic (or Rydberg) structure of the bands has been averaged out to produce a curve with a single maximum. This procedure seems justified, as the principal uses of such correlations are in confirmation of structure of new compounds or in the estimation of total concentration of a given type of compound in a complex mixture. Since the vibronic structure of the spectra varies appreciably within a given series, it tends to be averaged out in complex mixtures. On the other hand, it cannot be predicted either from empirical or theoretical considerations with enough certainty to be of value in the determination or confirmation of the structure of a new compound. However, the vibronic structure is more pronounced for compounds containing two or more alkyl substituents (Figures 5 and 6) on the ethylenic carbons than for the monoalkylethylenes (Figure 4). Within a given series there seems to be some tendency for the peak intensity to increase with increasing symmetry of the molecule. Thus it increases in the series: 4-methyl-*trans*-2-pentene < *trans*-2-hexene = *trans*-2-pentene < *trans*-2-butene = *trans*-3-hexene. Another effect has been pointed out by Carr and Stücklen (*8, 9*): a nearly linear relationship exists between the frequency of the absorption band having the longest wave length and the number of alkyl substituents on the ethylenic carbons. This bathochromic shift is greater for the cis than for the trans configuration.

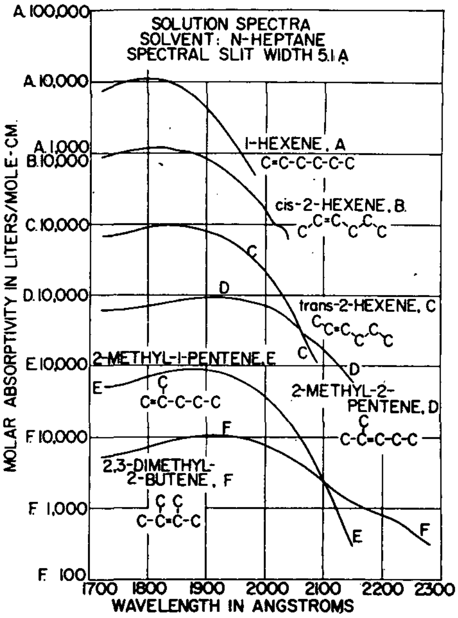

Figure 7. Far ultraviolet spectra of *n*-heptane solutions of representative hexenes

The spectra of *n*-heptane solutions of six of the hexenes are given in Figure 7. In going from vapor phase to solution spectra the peak intensity decreases slightly, a compensating increase in band width occurs and the bands shift about 40 A. to longer wave lengths. The integrated intensities of the bands remain virtually unchanged. Thus for 1-hexene the oscillator strengths, f,

$$f = 4.32 \times 10^{-9} \int \epsilon_\nu \, d\nu \qquad (3)$$

are computed to be 0.285 and 0.275 for vapor and solution spectra, respectively. In Equation 3 ν is the frequency in cm.$^{-1}$

It is obvious from examination of Figures 4 to 7 that the differences between the spectra of the individual olefins and between the average spectra of the various olefin classes are so small that the far ultraviolet spectra will be of very limited value in determining individual constituents of mixtures or even in estimating the relative total amounts of the olefin types. On the other hand, the similarity of all the spectra suggests that a total olefin determination may be possible even in very complex mixtures. At 1795 A. the average molar absorptivity of all of the olefins in the vapor state is 9800 with a standard deviation of 2680 and 95% confidence limits for the average of ±1200 or ±12.3%. The situation is more favorable if only the solution spectra are considered. Thus at 1860 A. the average molar absorptivity for the six hexenes is 9200 with a standard deviation of only 930 or 10.1%, while the 95% confidence limits for the average are ±9.6%. It is likely that this uncertainty will be reduced when more solution spectra are available. If interfering compounds are present the analysis of mixtures may require the use of another wave length where the difference between isomers is somewhat greater.

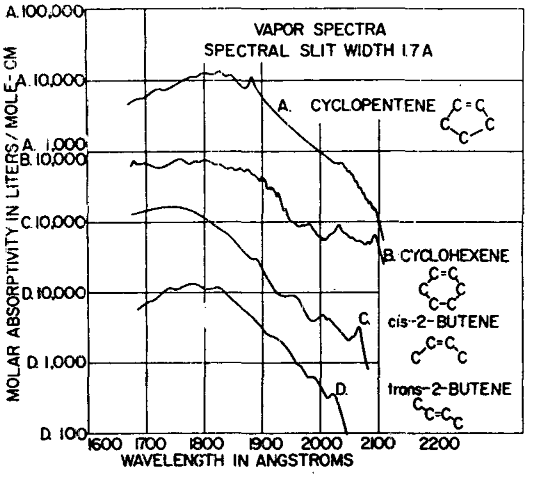

Figure 8. Comparison of far ultraviolet spectra of the cyclic and noncyclic mono-olefins

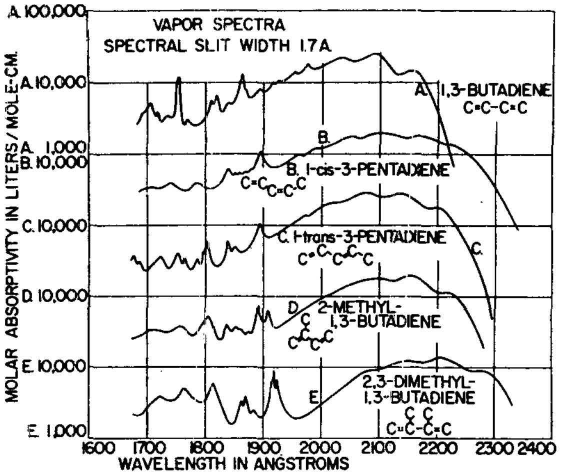

Figure 9. Far ultraviolet spectra of vapors of C_4 to C_6 conjugated diolefins

Cyclic Mono-olefins. The spectra of vapors of cyclopentene and cyclohexene are shown in Figure 8 along with those of *cis*- and *trans*-2-butene for comparison. Carr (*7*) has cited the similarity of the long wave-length bands of cyclohexene and of the *cis*-2-alkenes as evidence for the structure of the latter, since the cyclic compound can exist only in the cis configuration. Although more data will be required to reach definite conclusions, it appears from the spectra of the parent cyclic compounds that the total olefin determination suggested in the previous section would include the cyclic olefins as well as the alkenes.

Conjugated Diolefins. Figure 9 shows the vapor spectra of five of the conjugated diolefins. These compounds exhibit a broad, intense (ϵ about 20,000 to 28,000) band near 2150 A., analogous to the $N \rightarrow V$ band of the mono-olefins. It has a comparable vibronic structure and an integrated intensity about twice that of the corresponding band for the mono-olefins. This band has been used for the estimation of 1,3-butadiene in C_4-olefin mixtures and may have more general utility. In addition to these broad bands, there are also a number of sharp bands below 2000 A. which have been attributed to Rydberg transitions (*6*). Because these bands are sufficiently strong, sharp, and characteristic, they could be used to identify or estimate individual diolefins in fairly complex mixtures of hydrocarbons. The work of Jacobs and Platt (*14*) indicates, however, that this sharp structure is absent in the solution spectra, so that it would be necessary to use the vapor technique for such analyses.

Acetylenes and Allenes. Platt, Klevens, and Price (*24*) have measured the far ultraviolet spectra of *n*-heptane solutions of 1-octyne and 2-octyne and found a weak absorption band (ϵ about 150) at 2250 A. in each case. They also observed a stronger band (ϵ about 9500) at 1800 A. for the 2-octyne and steadily increasing absorption to 1700 A. with a step-out at 1850 A. (ϵ about 2000) for 1-octyne. The vapor spectrum of 1-butyne (Figure 10) is somewhat similar to Platt's results for the octynes with a step-out at 1850 A. and a maximum at 1720 A. (ϵ = 4500). Apparently the center of the absorption band system lies below 1700 A.

The spectra of three alkyl-substituted allenes are also shown in Figure 10. The monosubstituted compounds, 1,2-butadiene and 1,2-pentadiene, have a band at 1780 A. with ϵ about 20,000 and a step-out at 1880 A. There is also evidence of a third band below 1700 A. In the spectrum of the disubstitute allene, 2,3-pentadiene, all of these bands are shifted 30 or 40 A. to longer wave lengths and a new band appears at about 2000 A.

Nonconjugated Diolefins with Isolated Double Bonds. When the double bonds of these compounds are separated by two methylene groups, there is no interaction and the spectra resemble

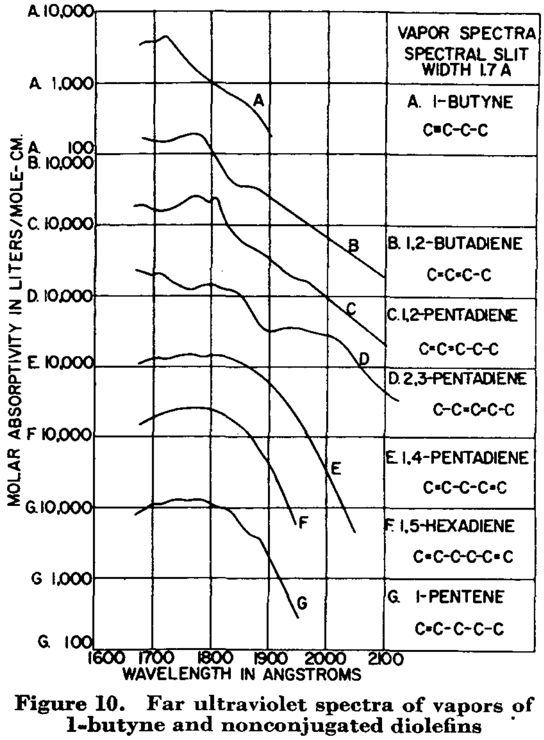

Figure 10. Far ultraviolet spectra of vapors of 1-butyne and nonconjugated diolefins

those of the corresponding mono-olefins except for a twofold gain in intensity. Thus the spectrum of 1,5-hexadiene (Figure 10) has the same contour as that of 1-pentene except for some loss of vibronic structure and the expected increase in intensity. On the other hand, a single methylene group does not provide complete "insulation" of the double bonds (*33*). Thus the absorption band system of 1,4-pentadiene is much broader than that of 1-pentene and the peak intensity is only about 40% greater.

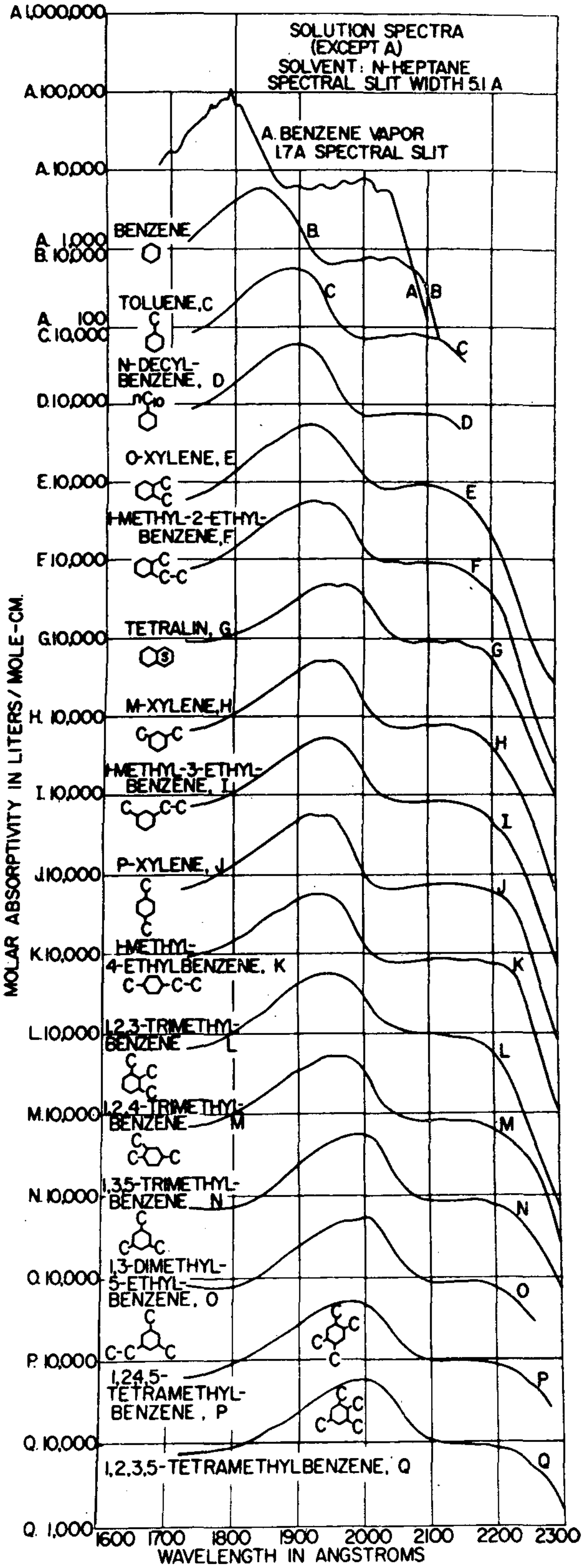

Figure 11. Far ultraviolet spectra of benzenes

Benzenes. The spectra of benzene, Tetralin, and 14 alkyl-substituted benzenes are presented in Figure 11. All of these compounds have two absorption bands between 1700 and 2200 A. The strong allowed π-electron transition [^1B in Platt's notation (*20*)] gives a band with maximum absorption at 1790 A. in the benzene vapor spectrum and at 1840 A. in the solution spectrum. It shifts to longer wave lengths with increasing alkyl substitution. A second transition, which has been described variously as a forbidden "free" π-electron transition (^1L_a in Platt's notation) and as a combination of such a forbidden transition with a Rydberg-type transition of a π-electron, results in a relatively flat absorption band about 150 A. wide between 2000 and 2200 A. for all of the benzenes. The maximum absorptivity of each of these bands is essentially unaffected by alkyl substitution as is shown in Table IV. The spread of only 15% in peak intensity of the ^1B band found in the present work is to be contrasted with a spread of nearly 50% in earlier papers (*21, 23*).

Table IV. Location and Intensity of Far Ultraviolet Absorption Bands of Benzenes in *n*-Heptane Solution

	^1B Band		^1L_a Band	
Compound	Wave length, A.	ϵ (center of band)	Wave length, A.	Peak, ϵ
Benzene	1840	60,000	1950–2100	7,900
Toluene	1890	56,000	1990–2130	8,100
n-Decylbenzene	1900	58,000	2000–2130	7,600
o-Xylene	1920	53,000	2030–2170	9,000
1-Methyl-2-ethylbenzene	1925	55,000	2030–2170	9,000
1,2,3,4-Tetrahydronaphthalene	1955	47,000	2050–2190	9,100
m-Xylene	1940	50,000	2040–2180	8,000
1-Methyl-3-ethylbenzene	1945	51,000	2040–2180	8,400
p-Xylene	1935	54,000	2030–2220	7,500
1-Methyl-4-ethylbenzene	1935	55,000	2030–2240	8,700
1,2,3-Trimethylbenzene	1950	55,000	2040–2200	10,000
1,2,4-Trimethylbenzene	1960	51,000	2040–2200	8,200
1,3,5-Trimethylbenzene	1985	55,000	2070–2220	8,800
1,3-Dimethyl-5-ethylbenzene	1985	50,000	2100–2200	9,100
1,2,3,5-Tetramethylbenzene	1995	55,000	2100–2240	9,100
1,2,4,5-Tetramethylbenzene	1975	50,000	2090–2220	9,600
Av.		53,000		8,680
Std. dev.		3,000		710
95% confidence limits of av.		±1,660		±390

These bands should be useful for determination of the benzenes. Thus total benzenes might well be determined at an appropriate position in the ^1L_a band region—for example, at 2110 A. where all the alkyl benzenes have very nearly identical molar absorptivities. On the other hand, the position of the ^1B band depends very largely on the number of alkyl substituents. A rough estimate of the relative concentrations of mono-, di-, and tri-, or more highly substituted benzenes could thus be made on the basis of measurements at wave lengths corresponding to the average positions of the ^1B band for these classes of compounds. This scheme will not be of high accuracy, however, as there is a considerable amount of overlapping of the ^1B bands for the various classes. The total alkyl benzene determination by this method will therefore be better than the breakdown according to number of substituents. Use of the ^1B bands rather than the ^1L_a bands will tend to minimize interferences in many cases, as these bands are among the most intense which have been observed for any chromophore in this region of the spectrum.

Catacondensed Aromatics. The far ultraviolet spectra of a number of catacondensed polynuclear aromatic hydrocarbons are given in Figure 12. From an analytical viewpoint one of the most interesting features of these spectra is the fact that none of these compounds has absorption bands in the 1800- to 2000-A. region which are as intense as the ^1B bands of the benzenes. This is in marked contrast to the situation in the near ultraviolet, where all of the polynuclear aromatics absorb much more strongly than the benzenes at all wave lengths. The naphthalenes have no clearly discernible bands in this region. Klevens and Platt

(*17*) have reported finding a band (^1B_a) at 1670 A., which was beyond the lower limit of the measurements used here, and another extremely weak band at about 1894 A. The present authors obtained a barely perceptible hump in the naphthalene spectrum at about 1900 A., but comparable bands cannot be found in the spectra of the monomethyl naphthalenes. This band is accentuated, however, in the spectrum of 1,2'-dinaphthyl and shifted to about 1950 A. In this compound steric hindrance between the hydrogens of the two rings prevents their being coplanar, so that effective conjugation of the rings probably does not occur. This is reflected in the negligible shift in position of the 2200-A. (^1B_b) band and in the generally strong resemblance of the 1,2'-dinaphthyl spectrum to those of the other naphthalenes.

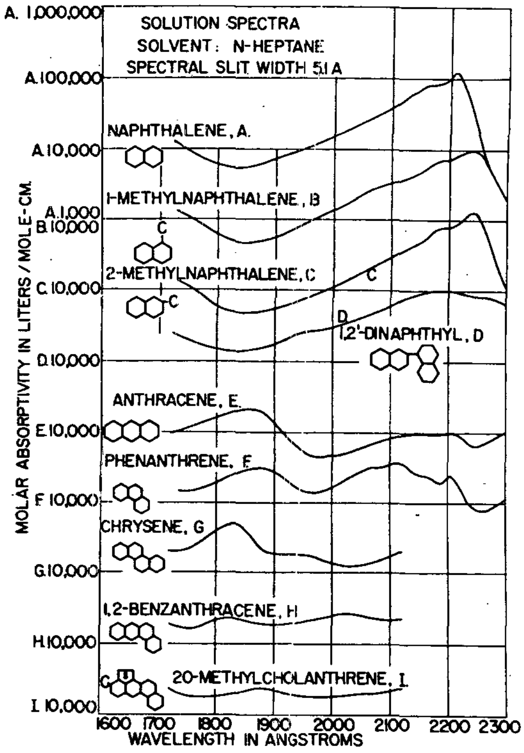

Figure 12. Far ultraviolet spectra of *n*-heptane solutions of catacondensed polynuclear aromatics

Platt (*20*) has pointed out that the spectra of all catacondensed aromatic compounds containing the same number of aromatic rings are similar with respect to the number and location of their absorption bands, although differences in the relative intensities of these bands will result from differences in molecular symmetry. These effects are demonstrated by the spectra of phenanthrene and anthracene. The ^1B_a band corresponding to an allowed transition involving a transversely polarized excited molecule occurs at 1855 A. for anthracene and at 1875 A. for phenanthrene. The peak molar absorptivities are of the same order of magnitude. On the other hand, the ^1C_b band (excited molecule with quadripole moment) of anthracene is "forbidden" by symmetry considerations, while that of phenanthrene is "allowed." Although the ^1C_b band of both compounds is centered near 2100 A., the peak intensity is only 9700 for anthracene compared to 37,000 for phenanthrene. Similarly, both chrysene and 1,2-benzanthracene have absorption bands (^1C_a) near 1830 A., although that of chrysene is more intense by a factor of over two. This band shifts 50 A. toward the red in the substituted benzanthracene, 20-methylcholanthrene. The ^1B_a band of all these compounds has a maximum intensity range of only 18,000 to 27,000 but exhibits rather large changes in position. It occurs at 1950 A. in chrysene, at 2020 A. in 1,2-benzanthracene, and at 2050 A. in 20-methylcholanthrene.

Saturated Hydrocarbons. The saturated hydrocarbons are, in general, transparent in this region and thus suitable for use as solvents. Purely for comparison with the foregoing data on unsaturated compounds the spectra of 2,2,4-trimethylpentane and *n*-decylcyclohexane are shown in Figure 13. More complete absorption spectra have been given by Pickett *et al.* (*19*) for cyclohexane and cyclopentane. Data on the short wave-length cutoffs of a number of paraffinic hydrocarbons have been given by Platt (*22*) and by Potts (*27*).

GENERAL OBSERVATIONS

In general the far ultraviolet spectra of solutions of all the unsaturated hydrocarbons may be characterized as consisting of very broad, nearly structureless bands. The spectra of all members of a given class of compounds are similar with respect to both position and intensity of their absorption bands. The far ultraviolet spectra may thus be expected to be of greater value in estimating the total concentration of all members of a group of compounds with similar electronic structure than they will be for distinguishing the various members of the group. This is perhaps fortunate, since other spectroscopic techniques are often well suited to the latter job but ill adapted to the former. The far ultraviolet method may be expected to be complementary to such methods as mass spectrometry and absorption spectrophotometry in the near ultraviolet and infrared.

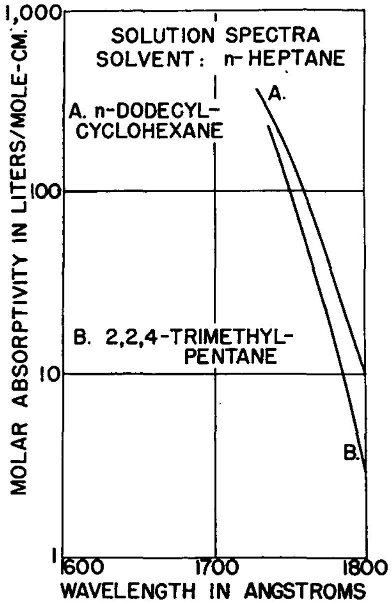

Figure 13. Far ultraviolet spectra of two saturated hydrocarbons

On the other hand, the vapor spectra of many compounds exhibit numerous sharp Rydberg lines which might be useful for the identification of individual members of a series, as was mentioned in the discussion of the spectra of the diolefins. For the most part these bands lie at shorter wave lengths (1000 to 1700 A.) than were covered by the present investigation. Quantitative measurements below 1700 A. may be made by known photoelectric techniques—e.g., (*39*)—but with considerably less convenience and probably with lower accuracy than is possible at longer wave lengths because of the greater effect of stray long wave-length radiation on the phosphor-sensitized phototubes which are generally used in this region. Also no stable source of continuous radiation is known for the 1000- to 1700-A. interval, so that the many-lined spectrum of hydrogen must be used. This places a serious limitation on the resolution and accuracy which may be obtained with a simple single-beam recording spectrometer. Reduction of the resulting records is also extremely tedious. Despite these experimental difficulties, the analytical potentialities of the shorter wave lengths appear promising enough to warrant exploratory work.

The primary purpose of the present paper is not to present finished analytical methods for the analysis of hydrocarbon mixtures but rather to stimulate an interest in analytical applications of far ultraviolet spectroscopy. The potentialities are large. However, many more spectra of pure compounds must be obtained before the utility of the method can be fully assessed, and instruments approaching the present recording spectrophotometers for the infrared and near ultraviolet in convenience and accuracy will be required before the method can be expected to achieve widespread acceptance.

ACKNOWLEDGMENT

The authors acknowledge the assistance of Arnold Friedman and E. L. Creamer, who obtained many of the data presented in this paper.

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RECEIVED for review July 17, 1954. Accepted October 30, 1954.

Determination of Hydrocarbons in Hydrogen by a Palladium Tube—Mass Spectrometer Method

R. A. BROWN, H. B. OGBURN, F. W. MELPOLDER, and W. S. YOUNG
Atlantic Refining Co., Philadelphia, Pa.

A convenient method is described for concentrating hydrocarbons in hydrogen-rich samples by pumping hydrogen through a palladium tube. By combining this procedure with mass spectrometric analyses 0 to 3% hydrocarbons can be determined individually with an error of 0.1% and a repeatability of 0.05%. Twenty minutes are required to obtain a hydrocarbon concentrate.

DETERMINATION of hydrocarbons in hydrogen-rich mixtures is currently of prime importance to the petroleum industry in naphtha catalytic reforming studies. In particular, the conclusions drawn from experiments performed in both laboratory and pilot scale equipment depend to a large extent upon accurate hydrocarbon determinations for such product streams. For units operated without gas recycle, an error of 0.1% in butanes in the gas analysis may mean an error of 5 to 15% or more, depending on the severity of operation, of the total butane production. This, in turn, yields an error for this component of about 0.7% in over-all material balance. The significance of this error in butane analysis on the gas stream is reduced in its effects when the experiment has been conducted in equipment with gas recycle. For this case, it would cause about 1 to 1.5% error in total butane production. Another serious problem is that small amounts of C_5 to C_7 components are not usually detected by a direct mass spectrometric analysis.

The best way to improve accuracy and detectability in such samples is to concentrate the hydrocarbons prior to analysis. This concentration consists of separating hydrocarbons from hydrogen by using palladium which is permeable to hydrogen but not to other gases (*4*).

Permeability increases rapidly with temperature. Fleiger (*3*) used this fact to determine hydrogen in mixtures containing methane, nitrogen, and carbon monoxide. In this case, the hydrogen content was based on amount of gas which would be pumped through a heated palladium tube. More recently Marshall and Constabaris (*5*) applied a palladium tube method to analysis of water gas mixtures. Based on this technique a method has been developed in which mass spectrometric analyses are obtained for samples depleted in hydrogen. This method has been in use for two years and appears to be vastly superior to a direct analysis.

APPARATUS AND PROCEDURE

The palladium tube as shown in Figure 1 has a wall thickness of 0.003 inch, an outside diameter of $^{19}/_{32}$ inch, and a length of 5 inches. The tube is silver soldered at a stainless steel ring connection to a Kovar tube which, in turn, is joined to glass by a graded seal. Teflon tape is wrapped around the stainless steel and Kovar to reduce the area of exposed metal surfaces. A glass coil is placed inside the palladium tube to prevent collapse of the tube under vacuum. This support was not part of the original apparatus and its presence would probably enable use of a tube having only a 0.002-inch wall thickness. Such a change would enhance the pumping out of hydrogen through the tube.

In use, these tubes become less permeable to hydrogen, apparently owing to occluded oxygen and/or sulfur. Normally, 2 minutes are required for a pump out but when a coating causes this to reach as high as 5 minutes, the tube is removed and polished with extra fine Carborundum, Grade H440. It is then ready for use again. Approximately 50 samples may be run on a tube before polishing is required. Palladium is particularly reactive to sulfur compounds and if hydrogen sulfide is known to be present, it is removed by passing the sample through Ascarite.

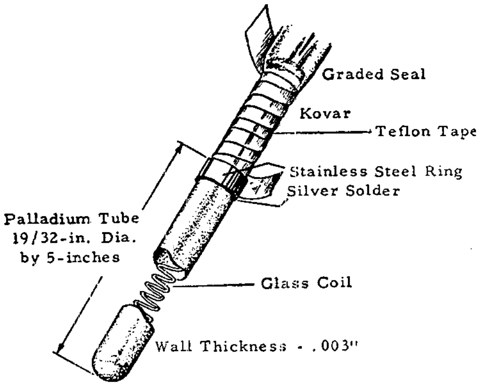

Figure 1. Palladium tube

The entire apparatus is shown in Figure 2. It consists of an oxygen analyzer, a sample reservoir, a palladium tube section, and apparatus for transfer of residual gas to a sintered disk tube for introduction to the mass spectrometer.

The apparatus is constructed so that the clearance between the palladium and outer glass tubing is about 0.1 inch. This outer glass tubing is kept at 500° F. The section containing the palladium tube is approximately 50 ml. in volume, most of which is shown as the glass bulb. A sample probe is located near the geometric center of the section which also includes a closed end manometer to indicate the amount of residual gas. The sampling section consists of a 100-ml. Toepler pump, T_2, and a point of attachment for a sintered disk tube (*6*).

In operation, a sample tube is attached at point *A*, Figure 2. The manifold is evacuated up to the stopcock of the Orsat tube and then 125 ml. of sample at atmospheric pressure are introduced into the Toepler, T_1. After oxygen has been determined by absorption in pyrogallol as measured by manometer M_1, the oxygen-free sample is forced into the palladium tube by displacement with mercury. During this displacement, stopcock S_1 is opened, so as to start immediately pumping hydrogen through the palladium. After about 92% of the sample has been pumped away as hydrogen, stopcock S_1 is closed, and the sample pressure allowed to equalize. At this point about 94% of the sample will have been removed. The residual gas, approximately 7.5 ml., is next expanded into the Toepler, T_2. Then, with Hoke valve (*1*) V_7 closed, sample is next displaced by mercury into a sintered disk tube.

Table I. Analysis of Hydrogen-Hydrocarbon Blend

Component	Mole % Known	Mole % Palladium tube mass. spec.
Hydrogen	99.978	99.964
Methane	0.011	0.016
Ethane	0.000	0.006
Propane	0.000	0.000
Isobutane	0.000	0.000
n-Butane	0.011	0.014
	100.000	100.000

Table II. Repeated Analysis of a Hydrogen-Hydrocarbon Mixture

Component	Mole % Mean of 8 Analyses	Mole % Std. dev.
Hydrogen	99.14	0.077
Methane	0.45	0.037
Ethane	0.008	...
Propane	0.013	...
Butenes	0.003	...
Isobutane	0.0005	...
n-Butane	0.32	0.036
Hexenes	0.0003	...
n-Hexane	0.064	0.016
Benzene	0.0003	...
	99.9991	

Table III. Analysis of Hydrogen-Hydrocarbon Blends

Component	Mole % Known	Mole % Mean	Mole % Standard error
	Fourteen Synthetic Samples		
Hydrogen	98.81	98.83	0.154
Methane	0.61	0.59	0.074
Ethane	0.01	0.01	0.006
Propane	0.00	0.01	0.015
Isobutane	0.00	0.01	0.009
n-Butane	0.57	0.55	0.102
	Three Synthetic Samples		
Hydrogen	97.02	96.999	0.21
Methane	1.455	1.47	0.082
Ethane	0.035	0.029	...
Propane	0.00	0.013	...
Isobutane	0.00	0.019	...
n-Butane	1.49	1.47	0.102
	100.000	100.000	

Oxygen is removed from the sample at the time of determination. This procedure serves two purposes. First of all, carbon dioxide and water may be formed in the presence of oxygen. Because the amount of this oxidation is variable, residual oxygen in the mass spectrometer sample cannot be correlated with that in the original sample. Thus, the true oxygen concentration must be determined prior to introducing the sample into the palladium tube. A second advantage results from removal of oxygen to minimize its occlusion by the palladium which reduces permeability to hydrogen.

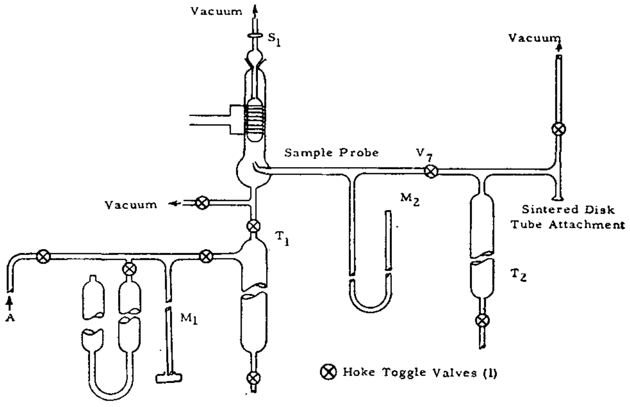

Figure 2. Palladium tube apparatus

Table IV. Determination of Hexanes

Component	Mole % Known	Palladium Tube–Mass Spec. 1	2	3
Propane	0.00	0.010	0.010	0.039
n-Hexane	0.226	0.19	0.22	0.19
Hexenes and/or C_6 cycloparaffins	0.00	0.006	0.006	0.007
Benzene	0.00	0.005	0.010	0.006
Sum[a]	0.226	0.206	0.241	0.223

[a] Sum represents equivalent molar concentration of *n*-hexane. Two moles of propane, 1 mole of hexene, or 1 mole of benzene are each equivalent to 1 mole of *n*-hexane.

CALCULATION AND RESULTS

To determine sample composition the amount of hydrogen pumped away must be known as well as composition and volume of the hydrocarbon concentrate. Volume of concentrate is determined by the manometer, M_2, and the known fixed volume of that section having the palladium tube. In expansion measurements this volume was found to be 50 ml. with the measurement made done with the palladium at 500° F. Since 125 ml. of sample at atmospheric pressure are used, the quantity of lost hydrogen can be obtained by a pressure-volume calculation. The hydrocarbon concentrate is calculated in a routine manner on an air-free basis. Nitrogen is corrected for the measured oxygen lost during its determination by absorption in pyrogallol.

The most important question concerning the validity of this method was whether or not representative samples could be collected for mass spectrometric analysis. Specifically, separation by molecular weight might occur in the palladium tube section and to study this possibility numerous synthetic blends of hydrogen, methane, and *n*-butane were analyzed. No separation was observed.

Results of one blend are shown in Table I. In this case 0.011% each of methane and *n*-butane was determined within 0.005%. This was the only experiment carried out in this concentration range and such results could probably be improved. In this case, 4 liters of sample were used to obtain a hydrocarbon concentrate.

Reproducibility is illustrated in Table II, where mean values for eight analyses of the same gas are presented. At concentrations of 0.064 to 0.45% standard deviations of 0.016 to 0.037% occur. Table III includes the mean analysis of 14 synthetic blends each made up separately to 98.81% hydrogen, 0.61% methane, and 0.57% *n*-butane. The standard error of 0.074% for methane and 0.102% for *n*-butane includes possible errors in synthesizing samples as well as analytical errors. Analyses of samples containing 97.02% hydrogen, 1.455% methane, 0.035% ethane, and 1.49% *n*-butane are also tabulated in this table. Here, a standard error of 0.082% occurred for methane and 0.102% for *n*-butane.

Samples containing hydrogen, methane, and *n*-butane indicated the ability of the method to determine hydrocarbons over the C_1 to C_7 range without loss due to handling.

A secondary problem was discovered during a study of synthetic blends containing small amounts of *n*-hexane. Results of such a study are shown in Table IV. Reference to these data shows that in addition to *n*-hexane, hexenes and/or carbon-6 cycloparaffins, benzene and propane were determined. These extraneous hydrocarbons are apparently the result of thermal decomposition (*2*) and represent about 10% of the *n*-hexane determined. These data were obtained with the palladium tube operated at 600° F. In an attempt to reduce this effect the operating temperature was lowered to 500° F. and Teflon tape was wrapped around the Kovar and stainless steel ring of the palladium tube assembly (Figure 1). The thermal decomposition for this arrangement appeared to remain about relative 10%, however. Palladium by itself at 500° F. was found to produce 5% decomposition, so that apparently no complete solution exists for this problem. However, a 10% decomposition of C_6 and C_7 components causes an insignificant error due to the low level of absolute concentrations encountered in samples. Furthermore, the decomposition products themselves are determined. No actual weight loss of total hydrocarbons occurs other than that due to the small amount of hydrogen formed during decomposition.

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RECEIVED for review August 24, 1954. Accepted November 17, 1954.

Colloidal Glass Suspensions for Use as Standards for Measurement of Thymol Turbidity

JOHN G. REINHOLD

The William Pepper Laboratory of Clinical Medicine, Hospital of the University of Pennsylvania, Philadelphia 4, Pa.

The suitability of colloidal glass suspensions for standardization of thymol turbidity measurements made by means of photoelectric spectrophotometers has been investigated. The preparation of a semipermanent standard resembling in its behavior the suspension formed in the thymol test together with its application to the calibration of photoelectric photometers for use in the thymol turbidity test is described. The readings of the turbid suspension produced by the reaction of the thymol-barbital reagent with blood serum vary in photoelectric photometers and spectrophotometers of different design. These variations are related in part to the composition of the serum.

THE lack of a satisfactory standard for measurement of thymol turbidity (*8*) in photoelectric photometers has caused considerable confusion. The use of barium sulfate suspensions for this purpose was proposed by Shank and Hoagland (*14*). Such suspensions are unstable and their reproducibility has been uncertain. A more serious difficulty arising in connection with the use of such standards was the erroneous description by Shank and Hoagland of the concentration of barium chloride used for preparation of the barium sulfate standard. This was said to be 0.0962*N*, whereas actually the corresponding molar concentration had been used.

Thus, at the time that measurement of thymol turbidity was adapted to photoelectric photometry two standards came into use. That consisting of barium sulfate prepared from 0.0962M barium chloride conformed to the readings obtained by use of the gelatin-formazine–egg albumin standards originally recommended by Maclagan (*8*). The second, prepared from 0.0962N barium chloride, gave thymol turbidity readings twice as high. The error was corrected, subsequently, but many who used this method remained unaware of the correction. Actually the majority in this country appear to be using standards based on the smaller units. For convenience, these have been called Shank-Hoagland units, to distinguish them from the units obtained by Maclagan's calculation.

Copper sulfate (*3*) and Evans blue (*7*) also have been used to some extent as standards. Both have the disadvantage of giving readings that depend directly upon the width of the spectral band as well as other optical characteristics of the photometer. These vary widely in the photometers ordinarily used in clinical laboratories. Readings of turbid suspensions are not nearly as dependent upon wave length. Moreover, the light intensity and the arrangement of the optical system have an important influence on turbidity readings. A suitable turbid suspension simulates more nearly the conditions affecting measurements of suspensions produced in a turbidity test than does a colored solution.

Glass suspensions have been in use for some time for serological and bacteriological studies as a means for estimating turbidities, and it appeared likely that they could be used for standardizing thymol turbidity measurements. Brewer and Cook (*1*) and later Hallinan (*4*) have described the preparation of bacteriological standards. Unfortunately, such colloidal suspensions vary widely in particle size and in their optical properties. The method proposed by Hallinan (*4*) for their standardization consisted of the preparation of successive dilutions of a colloidal suspension, from which larger particles had been removed by filtration through paper, to give an arbitrarily selected reading in a Klett-Summerson photoelectric colorimeter. Trial of this method proved it to be inadequate for standardization of thymol turbidity and other quantitative turbidity measurements currently used in clinical chemistry. The absorbances of such suspensions measured in various widely used spectrophotometers differed extensively and unpredictably and showed no consistent relationship to thymol turbidity.

Studies have been made of colloidal glass suspensions with the object of devising a method for obtaining a preparation suitable for use as a turbidity standard, particularly for thymol turbidity measurements. As a result of these studies, a colloidal glass suspension has been obtained that resembles in its behavior the turbid suspensions produced by reaction of serum with the thymol-barbital or certain other reagents. Its equivalence in terms of thymol turbidity has been established by several methods, and its reproducibility and other characteristics of its behavior have been investigated.

PROCEDURES

Thymol turbidity was measured 30 minutes after addition of 0.1 ml. of serum to 6 ml. of thymol-barbital reagent. In the earlier part of the study the reagent was prepared according to Maclagan (*8*); later a modified technique developed in this laboratory was substituted (*13*). The reagent was brought to 25° C. before addition of serum and was maintained within 0.5° of this temperature in a constant temperature during the 30-minute incubation period (*15*). Readings were made at room temperature which varied from 23° to 28° C. The thymol-barbital reagent was used for adjusting the photometer to zero.

Photometric readings were made by means of two Beckman Model DU spectrophotometers; two Evelyn photocolorimeters equipped with standard reflectors; a third Evelyn instrument with a dull mat reflector; and a fourth with a polished reflector as supplied by the makers for use with solutions of low transmittance; three Klett-Summerson photocolorimeters; and two Coleman, Jr., spectrophotometers. A few readings have been made using a Bausch and Lomb photoelectric spectrophotometer.

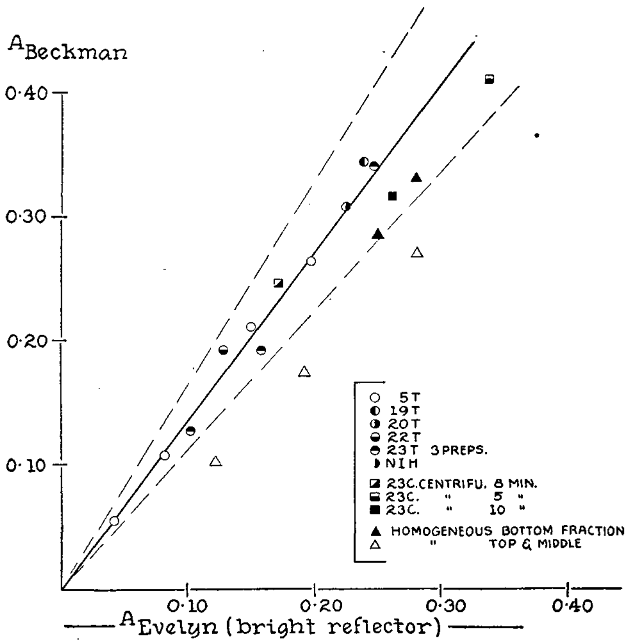

Figure 1. Absorbances of colloidal glass suspensions

Beckman spectrophotometer measurements compared with Evelyn photocolorimeter equipped with polished reflector

Table I. Effect of Preservatives on Stability of Colloidal Glass Suspensions

Days[a]	Preservative	Absorbance
0	Sodium benzoate, 0.1%	0.072
7		0.060
0	Merthiolate, 0.01%	0.131
9		0.086
0	Sodium borate, 1%	0.193
52		0.136
0	No preservative	0.193
52		0.192
0	Mercuric chloride, 0.01%	0.114
4		0.110
90		0.118

[a] Age of dilution of colloidal glass tested.

The Beckman spectrophotometer readings were made using 10-mm. cuvettes and the red-sensitive phototube. Slit width settings ranging from 0.04 to 0.30 had no effect on the measurements. Cuvettes of 20-mm. diameter were used in the Coleman, Jr., spectrophotometer, and in the Bausch and Lomb instrument these measured 18 mm. All instruments were adjusted to a zero reading with distilled water when glass suspensions were studied. Thymol turbidity measurements were made after the instrument had been adjusted to zero by means of a cuvette containing the reagent. All measurements were made at 660 mμ.

Results of photometric measurements are expressed either as absorbances or as Shank-Hoagland units of turbidity as defined above. The use of Shank-Hoagland units in preference to Maclagan units has been recommended by the Commission on Liver Disease of the Armed Forces Epidemiological Board as one step toward accomplishing a uniform system of reporting results of turbidity tests used in clinical laboratories.

EXPERIMENTAL

Preparation of Stock Colloidal Glass Suspension. A borosilicate glass reagent bottle of 1-liter capacity fitted with a glass stopper is filled to about one fourth its capacity with fragments of clean borosilicate glass. After being rinsed several times with distilled water, the glass fragments are covered with distilled water and agitated vigorously on a mechanical shaker until a milky suspension is produced. The time varies with the type of shaker, rate, and force of oscillation; 8 to 12 hours have been sufficient when a reciprocal shaker making an excursion of 8 inches 150 times a minute was used. The suspended glass is decanted into a cylinder of 1000-ml. capacity and diluted to the mark with distilled water. It is mixed and allowed to stand 72 hours. The uppermost 300 ml., which includes the more finely divided material, is decanted.

As an alternative method, the top 300 ml. may be divided among six conical centrifuge tubes of 50-ml. capacity. These are centrifuged at 1100 r.p.m. for 8 minutes in a centrifuge having a radius of 50 cm. The suspension is decanted into a clean borosilicate bottle fitted with a glass stopper.

Properties of Colloidal Glass Suspensions. STABILITY. The particles of glass settle very slowly, little sediment being visible on the bottom of the container after 24 hours. No change occurs during the time required for measurements of the absorbance.

PERMANENCE. The permanence of colloidal glass standards has been studied over periods ranging up to 18 months. In some preparations a decrease in turbidity, equivalent to 0.5 to 1% per month occurred during the first months. Others remained unchanged indefinitely. The growth of microorganisms can cause a rapid fall in absorbance. Addition of preservatives, with one exception, has accelerated deterioration (Table I), as has the presence of other solutes (Table II). The alkaline reaction of the suspensions of higher absorbance (0.30 upwards) inhibits growth and probably aids in stabilizing the suspensions. The use of a solution of mercuric chloride containing 0.1 gram per liter appears to have no deleterious effect on stability of dilute suspensions and yet checks growth. Information concerning stability of mercuric chloride-preserved standards over long periods is not yet available. Until such information is available, the use of freshly distilled water for preparing dilutions is recommended. Containers must be chemically clean.

The pH affects stability (Table II). Suspensions buffered at approximately neutral pH deteriorated slowly. At pH values ranging from 4.0 to 6.0, deterioration was rapid.

Fractionation of Colloidal Glass Suspensions and Selection of a Suitable Preparation. The method of preparation described by Hallinan (*4*) produces suspensions with a wide range of particle sizes. Such preparations when measured in different photometers or under different conditions of illumination varied considerably in their readings. Furthermore, their behavior differed from that of the suspensions produced by serum in the thymol test. It was not possible therefore to obtain satisfactory standards until preparations having a particle size range resembling the suspensions formed by the reaction of thymol-barbital with serum protein and lipides were isolated.

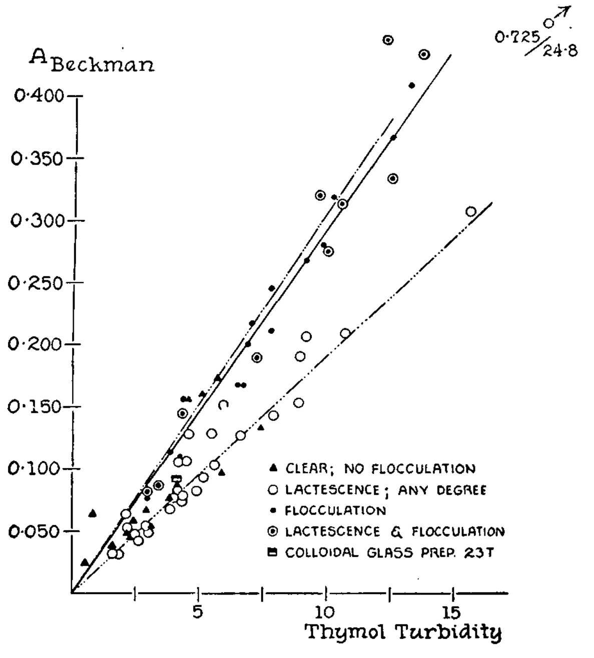

Figure 2. Influence of composition of serum upon absorbances of thymol turbidity tests

Clear and lactescent sera and sera that subsequently showed flocculation

Table II. Effect of pH and Various Buffers on Stability of Colloidal Glass Suspensions

Days[a]	Buffer	pH	Absorbance
4	None	6.9	0.139
11			0.136
0	Distilled water exposed to air 4 weeks	6.9	0.112
10			0.114
15			0.108
90			0.109
0	Freshly distilled water, low in CO_2	7.3	0.112
10			0.115
15			0.112
90			0.111
0	Phthalate, 0.05*M*	4.0	0.122
3			0.082
0	Acetate, 0.1*M*	5.0	0.131
3			0.103
0	Phosphate, 0.066*M*	6.0	0.135
3			0.117
0	Phosphate, 0.066*M*	6.9	0.109
10			0.105
15			0.102
90			0.088
0	Phosphate, 0.066*M*	7.4	0.109
10			0.102
15			0.094
90			0.086
0	Sodium borate, 0.05*M*	9.2	0.130
3			0.139

[a] Age of dilution of colloidal glass tested.

In order to accomplish this, glass suspensions were allowed to sediment in glass cylinders of 1-liter capacity. After 2 to 4 weeks, a series of zones, six to eight in number, each consisting of a fairly homogeneous range of particle sizes and varying in volume from 75 to 100 ml., could be distinguished. These zones were removed by aspiration and examined separately in several photometers. Their absorbances were compared with those of the turbid suspensions formed in the thymol turbidity test. Such evaluations were aided by the finding that the ratio of absorbance measured in the Evelyn photometer at 660 mμ to that measured in the Beckman Model DU spectrophotometer was determined by particle size.

Although numerous trials were made of individual zones removed at various levels from different preparations of colloidal glass, none of the separated zones resembled in its optical behavior that of the thymol-serum reaction product. This suggested that a variety of particles of differing sizes were being formed in the latter. Proceeding on this assumption, combinations of two and three separate zones were tested next. A satisfactory identity with the thymol suspensions was finally achieved by separating the upper 300 ml. of a glass suspension that had been allowed to sediment under the influence of gravity for approximately 72 hours. A similar preparation could be obtained much more rapidly by an alternative method, centrifugation at 1100 r.p.m. for 8 minutes as described above. However, the gravity method has provided more uniform preparations (Figure 1).

Figure 1 shows the relationship between the absorbances of a series of colloidal glass suspensions as measured on the Beckman spectrophotometer and an Evelyn photocolorimeter, the latter equipped with a polished reflector. The solid line shows the regression of gravity separated top fractions. The regression of the readings of thymol turbidities of specimens that subsequently showed flocculation is shown by the upper line; that of lactescent specimens, excepting those that flocculated, is shown by the lower line (Figure 2). Both the T (gravity separated) and C (centrifuged) series of glass suspensions fall between the two lines. Thirteen dilutions derived from eight different preparations are included. The greatest deviation in this group is about 5%, evidence that preparations can be reproduced successfully.

Several preparations derived by methods other than that yielding the T series are also shown (by open and solid triangles) in Figure 1. These include the middle and bottom cuts of preparation 23. Both depart from the relationship characteristic of the top fraction. A sample of a colloidal glass suspension (shown by the semicircle) prepared by the National Institutes of Health for standardization of vaccines and related applications, also departed from the limits defined by the thymol turbidity readings.

Heterogeneous Nature of Thymol Reaction and Significance for Measurement of Turbidity. The first attempts made in this study to establish a relationship between colloidal glass suspensions and thymol turbidity yielded disconcertingly variable results, especially when the thymol turbidity was measured by use of an Evelyn photocolorimeter equipped with a bright reflector. Ratios of thymol turbidity to Beckman readings ranged from 65 to 18 (Figures 2 and 5). Similar relationships were seen when certain other photometers were used. The possibility that these differences in optical behavior were related to reaction of the several serum constituents known to contribute to the turbidity was investigated. It is well established that in addition to gamma globulin [Maclagan and Bunn (*10*)] and β-globulin [Recant, Chargaff, and Hanger (*11*); Cohen and Thompson (*2*)], the lipides of serum react with the thymol barbital reagent to cause turbidity [Kunkel and Hoagland (*6*)].

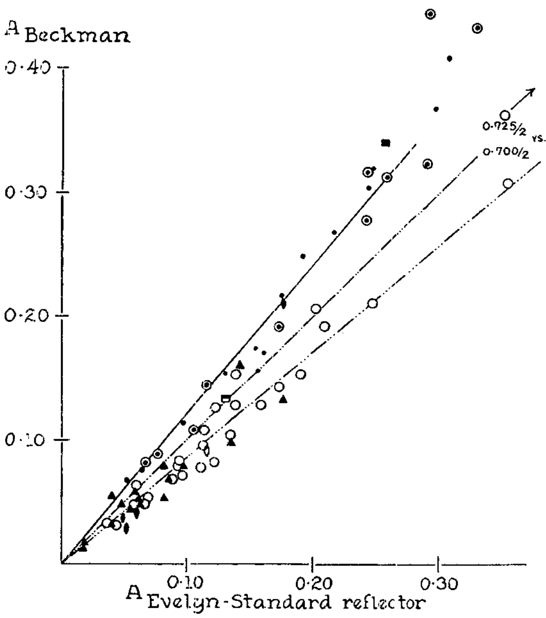

Figure 3. Distribution of thymol turbidity readings

Readings with Evelyn photometer equipped with standard reflector plotted against Beckman spectrophotometer readings. Symbols have same meaning as in Figure 2. Ovals represent thymol turbidities done in buffer of pH 7.80

In order to test the effect of a predominance of a reaction with the protein components in certain sera as compared with predominance of lipide reaction in others, thymol turbidities of sera that subsequently showed flocculation on standing with the thymol-barbital reagent were measured in both photometers. The ratios of absorbances plotted as shown in Figure 2 fell along the upper broken line. The thymol turbidity readings of lactescent sera, known to be high in lipide, were plotted in the same way. The ratios of the latter fell along a line of considerable less slope, the lower line in Figure 2. Some, but not much overlapping of the flocculation group occurred. When lactescence was associated with flocculation, the latter determined the location of the point of the graph. Clear sera that did not show flocculation gave ratios that fell between those of the flocculent and lactescent specimens, although much closer to the former, as shown by the solid line in Figure 2. Table III shows the equations for the regression of the three groups of measurements calculated by the method of least squares. The regression coefficients of the lactescent and flocculent groups tested statistically differed significantly.

Table III. Regression Coefficients of Different Groups of Turbidity Readings[a]

Colloidal glass suspensions	$TT = 40\ A + 0.1$
Lactescent sera	$TT = 51.9\ A + 0.2$
Flocculent sera	$TT = 30.7\ A + 0.7$

[a] TT = thymol turbidity, A = absorbance measured in Beckman Mode DU spectrophotometer.

Readings made with the Beckman spectrophotometer when compared with those of Evelyn photometers (equipped with standard reflectors) and by means of Coleman, Jr., spectrophotometers showed a similar spread, again depending upon the composition of the serum (Figures 3 and 4). In Figure 3, the regression of flocculent sera fell along the upper line, as did that of lactescent sera that gave rise to flocculation. The regression of sera either clear or lactescent that did not produce flocculation fell along the lower broken line. A further decrease in light intensity in the Evelyn instrument brought about by the use of a dull mat reflector had little effect on the readings.

The colloidal glass preparation selected for use as standard was one that when tested by this method gave readings that appeared on the graph midway between the regression lines of the flocculating and lactescent sera as shown by the broken line with the two dots between dashes in Figures 3 and 4. The solid square shows readings of the National Institutes of Health's colloidal glass suspension, and the partially shaded squares those of preparation 23T.

The difference between the thymol turbidity readings of the two types of sera is frequently sufficiently large and consistent to enable the thymol turbidities predominantly caused by change in protein (the flocculation type), to be distinguished from those apparently due to elevation of lipide (the lactescent type). It is probable that these may differ in clinical significance as well as chemical. Figure 5 shows the frequencies with which ratios of thymol turbidity to absorbance occurred in "flocculating" as

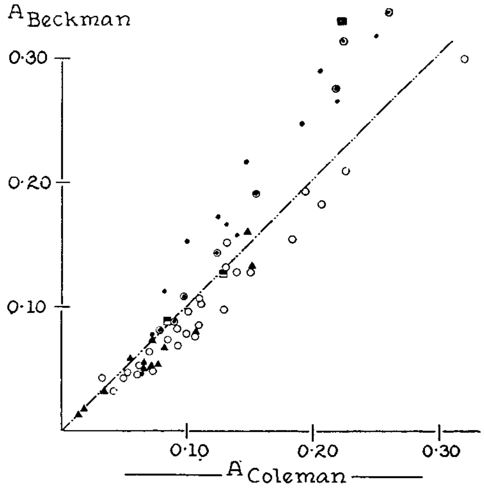

Figure 4. Influence of serum composition on absorbance

Same relationships as in Figures 2 and 3, except that Coleman, Jr., spectrophotometer used to measure absorbances

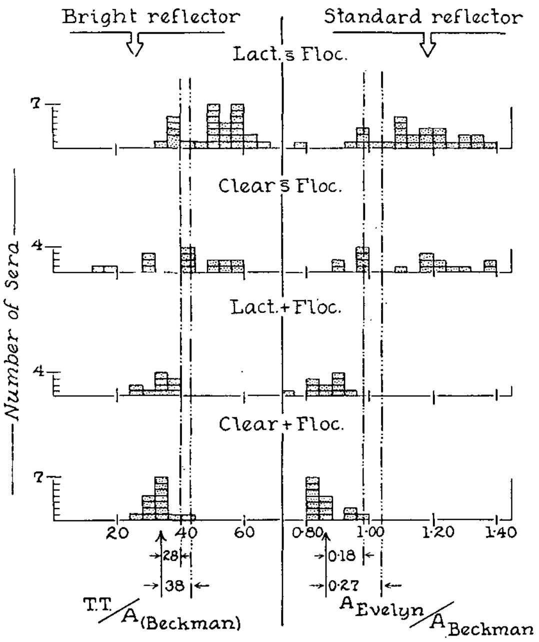

Figure 5. Frequency of occurrence of given ratios of thymol turbidities to absorbances in different types of sera

compared with lactescent sera. The former group is homogeneous, but in the "lactescent" and "clear" groups that failed to show flocculation the distribution is bimodal. This finding suggests that low ratios should be regarded as an indication of abnormality of the type associated with liver disease. Present methods of evaluating the thymol test fail to demonstrate such abnormality in many specimens. Further studies of the clinical potentialities of this observation are planned.

Standardization of Colloidal Glass Suspensions. The absorbance of the stock glass suspension is measured by using a wave length of 660 mμ. Water is used for adjusting the photometer to zero. If the absorbance exceeds 0.30, it is diluted with freshly distilled water sufficiently to give an absorbance of approximately 0.15. The cuvette should be similar in its optical characteristics to those used for the thymol or other tests. The quality of the distilled water used for diluting the concentrated suspensions may be important. Freshly distilled water is preferred to deionized water.

Many different types of photoelectric photometers are currently used in clinical chemistry, and one of the necessities for a suitable standard is that it give similar results in several, at least, of the more widely used instruments. Tests made of a group of photometers indicated that absorbance measurements agreed in some but differed significantly in others. The differences between Beckman DU spectrophotometer readings and those of Evelyn and Coleman, Jr., photometers have been described; such differences were related in part to the composition of the serum and type of reaction.

The relationship between absorbance of the colloidal glass suspensions prepared by the technique described above and their equivalence in units of turbidity can be derived from Figures 2, 6, and 7 and Table IV.

The thymol turbidity readings used for these calibrations were obtained by means of an Evelyn photocolorimeter equipped with a polished reflector. This instrument was calibrated by equating the results of some 500 readings made by the method originally proposed by Maclagan (*8*), the Kingsbury, Clark, Williams, and Post turbidity standards for estimation of urine protein, with the absorbances obtained by use of the Evelyn instrument. The equivalent of the urine protein reading obtained from the Kingsbury standards was divided by 60 to obtain thymol turbidity in Maclagan units. This calibration was verified by averaging the readings of 25 different preparations of barium sulfate prepared according to the directions of Shank and Hoagland (*14*); 0.0962*M* barium chloride was used. The results of the two methods of calibration, doubled in order to obtain Shank-Hoagland units, were identical. The resulting calibration gave values 5% lower than those derived by calculation of the mode of the measurements of a suspension of colloidal glass made by 45 different laboratories (*12*). This is not a significant difference.

Table IV. Average Ratios of Thymol Turbidity to Absorbance with Several Spectrophotometers

	No. of Specimens	TT/A Mean	TT/A Range
Beckman, DU	69	45.2	27–60
Coleman, Jr.	66	44.1	32–54
Evelyn, standard reflector	69	42.4	34–49
Evelyn, polished reflector	62	58.7	52–61
Klett-Summerson	41	0.137	0.10–0.22

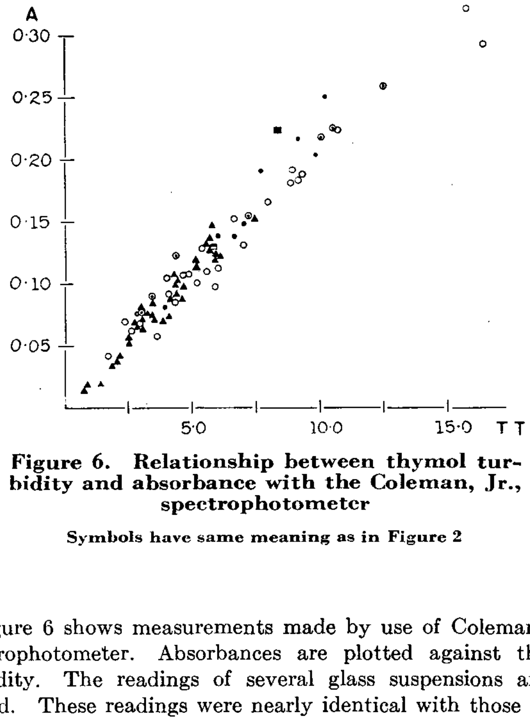

Figure 6. Relationship between thymol turbidity and absorbance with the Coleman, Jr., spectrophotometer

Symbols have same meaning as in Figure 2

Figure 6 shows measurements made by use of Coleman, Jr., spectrophotometer. Absorbances are plotted against thymol turbidity. The readings of several glass suspensions are included. These readings were nearly identical with those found by use of the Evelyn instrument.

Table IV shows the averaged values of the ratio of thymol turbidity to absorbance of 41 to 69 specimens of serum together with the range of variation measured by instruments of different design. The factors in Table IV were obtained in each instance by use of several instruments of a given make, and the ratios of thymol turbidity and absorbance, therefore, may be regarded as representative. Thymol turbidity may be calculated by multiplying the absorbance by the ratio of thymol turbidity to absorbance. However, it is advisable to calibrate each instrument by means of a standard colloidal glass suspension rather than to depend on factors derived in other laboratories.

When a glass suspension is used as a standard, thymol turbidity is calculated as follows: $T_s = A_s \frac{T_m}{A_m}$ where T_s represents the units of turbidity of the glass suspension, A_s its absorbance at 660 mμ, and T_m/A_m the mean ratio of thymol turbidities to absorbances tabulated in Table IV.

To obtain the thymol turbidity, TT, of a serum specimen, the following calculation is used:

$$TT = A\frac{T_s}{A_s}$$

where A is the absorbance of the turbid suspension produced by reaction of serum with thymol-barbital reagent and T_s and A_s have the same significance as in the preceding equation.

The regression equations calculated for the values in Figures 1 to 4 and 6 and 7 indicate that the origins are sufficiently close to zero to allow intercepts to be disregarded.

Thymol-barbital reagent is used to establish the zero setting when thymol turbidity is measured, but water is used for this setting when the glass suspensions are read.

The influence of changing the optical system in the Evelyn photometer is shown in Figure 7. Thymol turbidities expressed as absorbances measured in an Evelyn photometer equipped with a polished reflector are plotted against absorbances measured in a similar instrument with an unpolished reflector of the type supplied as standard equipment. The absorbances obtained by use of the bright reflector are approximately three fourths those of the standard instrument. However, the colloidal glass suspension was affected nearly to the same extent as were the thymol turbidities, so that measurements referred to the glass standard would have differed by no more than 10%. There is little difference between the behavior of lactescent sera as compared with flocculating sera produced by this alteration in the Evelyn instrument.

DISCUSSION

The results of photometric measurements of thymol turbidity will vary, depending upon the light intensity and the arrangement of the optical system of the photometer used. The extent of the effect upon the readings varies in different instruments. Thus, the Evelyn photoelectric colorimeter and the Coleman, Jr., spectrophotometer gave similar results for thymol turbidity readings. The Klett-Summerson photoelectric colorimeter and Beckman Model DU spectrophotometer gave results that often differed from those of the other two instruments. These differences, particularly of the Beckman instrument readings, depended upon the predominance of the reaction of protein as opposed to lipide components of serum with the thymol-barbital reagent.

The importance of the instrument used for photometric readings of thymol turbidity has been pointed out by Hoyer and Jorgensen (*5*). However, the shortcomings of visual measurements of thymol turbidity are far greater than those of photometric (*12*).

The values derived from readings of standards used for calibration of the photometer or for direct comparison in visual measurements also are affected by the instrument or, in the case of visual comparison, by the light intensity and the positions of light, samples, and observer. This explains, in part, the large differences found between the results of different laboratories (*12*) despite the use of a standard such as barium sulfate. However, the well-known difficulties of reproducing suspensions of this substance are undoubtedly the most important cause of such variations.

Maclagan (*9*) has replaced the egg albumin–formazine-gelatin standards originally proposed with a standard prepared by treatment of diluted serum of known protein content with sulfosalicylic acid. The author lacks sufficient experience with this standard to enable evaluation of its reproducibility or conformity of the particles produced to the size and surface area of those produced in the thymol test. Variations arising from differences in compositions of the serum and technique of combining serum and reagent are to be expected. Such a standard also lacks permanence.

The suspension of colloidal glass described in this paper offers the advantages of semipermanence and of resembling more nearly than other standards in general use the particle size and optical behavior of the turbid suspension produced in the thymol test. The suspensions of colloidal glass diluted with 0.01% mercuric chloride solution have remained unchanged for one year. The use of this solution rather than distilled water therefore is recommended for dilution of the stock suspension of colloidal glass.

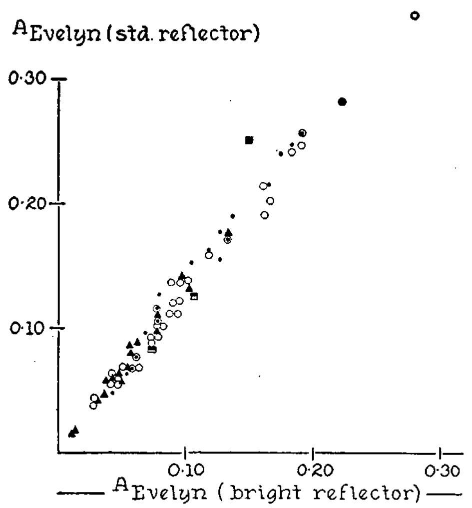

Figure 7. Absorbances of thymol turbidities

Measurements with Evelyn photometer equipped with standard reflector compared with those found by similar instrument with standard reflector

ACKNOWLEDGMENT

The author is indebted to Charles Jones, Veterans Hospital, New Orleans, La., for the suggestion that colloidal glass suspensions might be utilized for standardization of thymol turbidity readings, and to Roderick Murray, Division of Biologics Control, National Institutes of Health, Bethesda, Md., for a sample of a colloidal glass suspension prepared by the National Institutes of Health.

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RECEIVED for review July 15, 1954. Accepted October 25, 1954. Presented in part before the Division of Biological Chemistry at the 121st Meeting of the AMERICAN CHEMICAL SOCIETY, Milwaukee, Wis., April 1952. Work sponsored by the Commission on Liver Disease of the Armed Forces Epidemiological Board and supported by the Office of the Surgeon General, Department of the Army.

Continuous Detection and Measurement of Low Concentrations of Oxygen in Gases

LEONARD P. PEPKOWITZ

Knolls Atomic Power Laboratory, General Electric Co., Schenectady, N. Y.

The continuous detection and measurement of low concentrations of oxygen in gases is of paramount importance in liquid metal heat transfer technology. This paper describes an instrument based on the dew point principle for such continuous detection and measurement. A sensitivity of at least 0.0005 volume % of oxygen is attained with a mean deviation for precision of ±0.0001 volume %. The instrument is applicable to the determination of oxygen in the common gases such as nitrogen, helium, the other rare gases, hydrogen, and carbon dioxide.

ONE of the main difficulties in the use of liquid alkali metals as heat transfer agents is that of preventing oxygen contamination of the system. The resulting sodium monoxide causes increased corrosion, in addition to plugging the system. As this is a cumulative effect, exacting control of the oxygen content of the inert gas blankets is required on a continuous basis. Because of the cumulative effect, the oxygen concentrations must be held to very low limits, oftentimes below 0.001 volume % on systems which must operate for long periods of time.

Although a sensitive and quantitative batch method for such analyses has recently been described (*3*), ideally, a continuous monitoring instrument is required.

In contemplating the design of such an instrument, a consideration of the use of the data can simplify the problem. In almost all cases, the analytical data are used to assure the operators of the system that the oxygen concentration of the inert gas is equal to or below a predetermined specification value. If the oxygen concentration rises above this preset value, corrective measures must be taken. Thus the data of interest are not required to be absolute in the usual analytical chemical sense, but the measurement required is the deviation from the specification value.

Excess oxygen in the inert gas is usually caused by a breakdown of the purification system or a leak in the apparatus. This requires immediate remedial action by the operators. Conversely, the fluctuations in the oxygen concentration below the design value should not actuate the instrument and be the cause of repetitive false alarms.

The available instrumental methods for the continuous measurement of oxygen concentration in gas—such as magnetic susceptibility, and measurement of the heat of reaction with hydrogen or polarographic techniques—are not applicable in this range (below 0.005%) because of lack of sensitivity or reproducibility. However, the application of the dew point principle is ideally suited to meet the above mentioned objectives. Other chemical methods applicable to this range are adequately reviewed by Pepkowitz and Shirley (*3*).

The application of dew point methods to absolute measurements is limited by such considerations (*2*) as the temperature gradient across the gas-liquid interface, inherent slowness of the diffusional process, and the hysteresis effect in the dew evaporation. On the other hand, the method possesses relative simplicity, great sensitivity, and applicability to gases under flow conditions at atmospheric pressure or above.

The major advantage of the dew point method, besides its application to continuous detection, resides in its applicability to differential measurement. The temperature of the mirror can be set at any desired dew point corresponding to the specification concentration of oxygen in the gas when converted to water. At all moisture concentrations below this value, the mirror will remain clear and the fluctuations below this level will not actuate the alarm system. However, when the moisture concentration rises above this value, frost will form immediately and the instrument will actuate the alarm circuit. Thus the sensitivity of the apparatus is maintained over an extended range of concentrations limited only by the rate of diffusion of the moisture in the gas to the mirror and the instrument response time. The instrumental response is not limiting even at very low moisture concentrations because of the sensitive photoelectric system utilized (*1*).

APPARATUS

The continuous oxygen detector was constructed by additions to and modifications of the commercially available General Electric continuous dew point recorder (Catalog No. 32C409G15). The operation of the photoelectric system, thyratron unit, refrigeration system, etc., has been adequately described (*1*).

The modifications and additions are:

1. Installation of a multicircuit switch (Mallory 3263-J) which enables the instrument to function normally as a dew point recorder in position 1 (dew point) or when turned to position 3 (oxygen) makes the following circuit alterations to convert the instrument to the continuous oxygen detector.

Connects the mirror heater to an external Variac controlled circuit and disconnects it from the thyratron unit, switches the thyratron output to the alarm socket, and disconnects the automatic hourly defrosting switch.

2. Addition of an external 110-volt Variac-controlled circuit to energize and control the mirror heater.
3. Addition of a 110-volt Variac-controlled circuit to energize and control the platinum catalyst heating furnace.
4. Installation of an indicating pyrometer for the catalyst heating furnace.
5. Installation of the gas inlet system including valves, hydrogen introduction system, and platinum catalyst assembly described below.

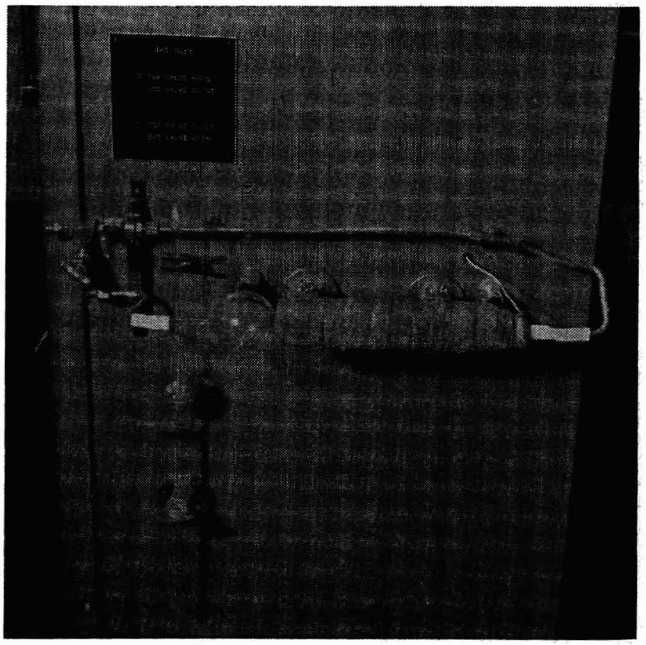

Figure 1. Gas inlet and catalyst assembly

The gas inlet system is comprised of a by-pass valve assembly, a one-way hydrogen metering and injection system, and a heated platinum catalyst section. The details are shown in Figure 1. The two-valve arrangement on the copper inlet line allows the test gas to pass directly into the instrument when operating as a dew point recorder (top valve open, bottom valve closed) or passes the gas through the hydrogen inlet section and the catalyst section (top valve closed, bottom valve open). The glass stopcock at the bottom of the mercury valve assembly is for initial purging to remove oxygen from the hydrogen metering system.

The simple one-way mercury valve system was used to introduce hydrogen from a No. 4 cylinder of extra-dry grade hydrogen as the gas source. At the very slow rate of addition after the initial purging, a No. 4 cylinder will last for many months. The one-way mercury valve system also helps to control the effect of the back pressure of the test gas and acts as a simple flowmeter. This is accomplished by regulating the gas flow to approximately 10 bubbles of hydrogen per minute. In actual practice this very simple arrangement proved to be reliable for long periods of time after the initial adjustment and the maintenance of a steady pressure of the test gas.

At the very low concentrations of oxygen and hydrogen involved, the conversion to water will proceed only in the presence of a catalyst. Accordingly, the heated platinum catalyst section was incorporated into the gas inlet system as shown in Figure 1. This has proved effective even in the range of oxygen concentrations below 0.001%. The connection between the glass tubing and the copper system at the exit end of the heated section is made with silicone rubber tubing which will withstand the elevated (150° C.) temperature. All other glass-to-metal connections are butt joints made with short lengths of Tygon tubing.

Although in actual practice the alarm outlet is connected to an external alarm system during the development work, the change in oxygen concentration was followed by noting the light intensity of a 60-watt bulb plugged into the alarm outlet. This proved to be very convenient, as the rate of dew deposition was indicated by the change in light intensity of the bulb. For actual records, a recording ammeter with a suitable series resistance was used.

Figure 3 shows a front view of the instrument with the dew point cell and phototube assembly visible. Figure 4 is a view of the left side of the instrument showing the auxiliary heating circuits, pyrometer, selection switch, and alarm outlet.

OPERATION OF INSTRUMENT

Start the refrigerator units by following instructions (*1*), and energize the heater circuit for the platinum catalyst furnace. While the refrigerator is cooling the mirror, adjust the temperature of the furnace to 150° C. Close the top valve and open the bottom valve in the gas inlet system.

With the selector switch on dew point follow the operating instructions (*1*). Set the sample gas flow at approximately 0.1 cu. foot per minute (0.05 cu. foot per minute for helium). Introduce hydrogen at approximately 10 bubbles per minute through the mercury seal on top of the fritted glass disk.

Record the dew point until the lines are purged and a reproducible dew-point record is attained. When a steady state is realized, turn the selector switch to oxygen.

By successive adjustment of the mirror heating control on the left side of the cabinet, adjust the mirror temperature to the reference dew-point temperature. This is based on the allowable oxygen concentration in the test gas and can be ascertained from the dew-point table (*1*), remembering that each mole of oxygen will be equivalent to 2 moles of water, so that the values in the table must be divided by 2.

When a steady state has been attained, the mirror should remain clear, unless the gas is higher in total oxygen than allowed.

To use the instrument as a straight dew-point recorder, turn selector switch to dew point, open the top valve, close the bottom valve of the gas inlet system, and follow instruction in (*1*).

To determine the approximate total oxygen content of the gas including moisture, place selector switch at dew point, and measure dew point of gas plus added hydrogen passed over the heated catalyst. The difference between the straight dew-point value and this value is equivalent to the oxygen concentration in the gas.

RESULTS AND DISCUSSION

The first tests, after the flow conditions for the gases and the temperature of the catalyst had been ascertained, were to pass helium containing 0.001% of oxygen through the apparatus with the mirror temperature set at −50° F. (dew point equivalent to 0.021% oxygen). Nitrogen from the general laboratory supply was passed into the instrument through a three-way stopcock. This gas contained $\sim$0.03% of oxygen as the sum of the oxygen and moisture in the gas. The response of the instrument was followed by noting the response time and the brilliance of the 60-watt light bulb plugged into the alarm socket. The response was practically instantaneous and the brilliance was equal to the normal output on the usual 110-volt circuit.

When the gas supply was switched back to the cylinder of helium, the light emission decreased as the frost on the mirror evaporated back into the dry gas. After some 5 minutes, depending on the amount of frost that accumulated on the mirror, the output signal was back to zero and the mirror was clear. As the last traces of frost were evaporated, there was a continuous decrease in the light emission of the bulb. The sequence could be repeated at will by switching from one gas supply to the other.

To determine the sensitivity and response characteristics of the instrument, various gases contained in standard cylinders were tested by first determining the straight dew point of the unknown gas and then adjusting the mirror temperature to 1° to 2° above this value with the selection switch turned to oxygen. The recording microammeter was connected to the alarm outlet and the pen was adjusted to zero. When a steady state was attained, hydrogen was introduced at the preset rate. Within a few seconds, the milliammeter needle indicated the presence of oxygen. When the pen approached the top of its excursion (usually off scale) the hydrogen flow was stopped and after a variable period of time depending on the oxygen concentration in the gas, the pen returned to or approached the initial setting.

A number of such tracings are shown in Figure 2. The hydrogen was introduced at point *A* and turned off at point *B*. The rate of rise of the milliammeter tracing indicates the response time; however, in some cases the instrument was switched off

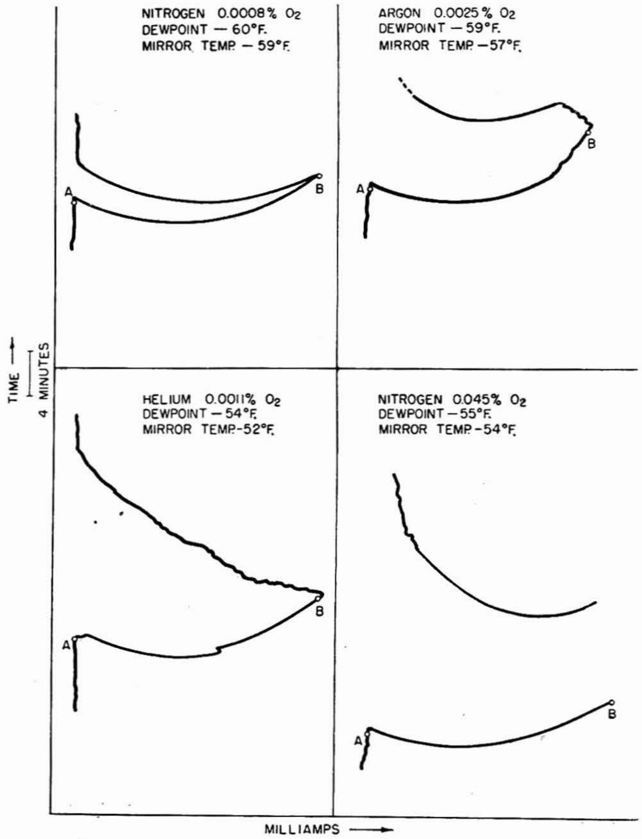

Figure 2. Response characteristics and sensitivity of continuous oxygen detector

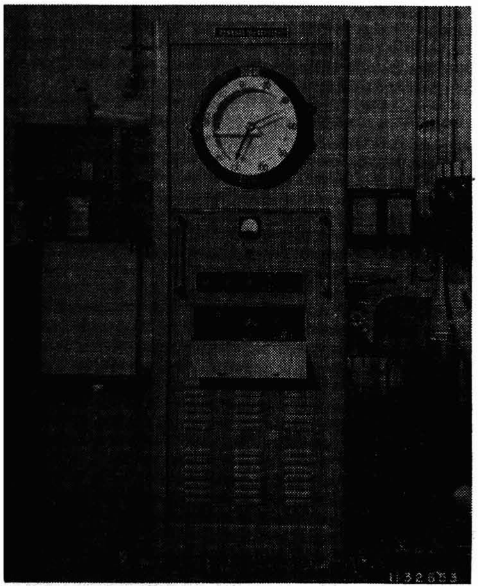

Figure 3. Front view of oxygen analyzer

near the top of the excursion to save the pen from injury when hitting the stop.

The response time of the equipment was very rapid. Although the test gas had to pass through some 5 feet of tubing, the instrument responded within 5 seconds after the introduction of the hydrogen.

The recovery time after stopping the hydrogen flow was a function of the oxygen concentration in the gas and flow rate. In the lower concentration the recovery was relatively very rapid, but at the higher concentrations of oxygen it tailed off more slowly, as shown in Figure 2. In the high oxygen concentrations much more water was frozen out on the mirror and the hysteresis effect is noticeable. At the lower concentrations the thin frost films evaporate rapidly, minimizing the hysteresis effect and increasing the recovery rate.

Table I. Oxygen Concentration in Test Gases

Cylinder No.	Gas	Dew Point of Gas	Dew Point of Gas + H_2	Δ H_2O, Vol. %	O_2[a], Vol. %	O_2[b], Vol. %
K-989247	N_2	−60	−56	0.0016	0.0008	0.0008
H-669	N_2	−55	− 9	0.0911	0.0456	0.0523
807768	A	−59	−55	0.0017	0.0009	0.0010
251242	A	−59	−49	0.0050	0.0025	0.0011
544	He	−54	−50	0.0022	0.0011	0.0011

[a] This paper.
[b] Method of Pepkowitz and Shirley (*3*).

The effect of flow rate is indicated in the response curve for helium (Figure 2). Because of the higher heat capacity of helium the flow rate is reduced to 0.05 cu. foot per minute to avoid heating the mirror. The slower recovery time as shown in Figure 2 reflects this effect, as the rate of frost removal from the mirror is decreased because of the smaller volume of gas passing over it.

The sensitivity of the instrument can be set very conservatively at 0.0005 volume % of oxygen. Although the lowest concentration indicated in Figure 2 is 0.0008% by volume, a full scale deflection was obtained. As no lower concentration of oxygen was available to ascertain directly the sensitivity of the instrument and assuming that there may be a threshold value required for the reaction of oxygen with hydrogen under the described flow and temperature conditions, the 0.0005% sensitivity is claimed.

The oxygen values detected and subsequently measured were in the presence of approximately 0.01% moisture in the sample gases. This emphasizes the inherent sensitivity of the instrument and dew-point methods in general. In the range of interest (less than 0.01% oxygen) the average equivalent of the 1° F. differential, which can easily be attained, is approximately 0.0001% oxygen, and the oxygen equivalent decreases as the dew-point value of the test gas is lowered. Thus at 0.001% (−85° F.), the 1° F. differential is equivalent to 0.00003% of oxygen. It is one of the attributes of the dew-point method that the sensitivity as a function of temperature increases with a decrease in the dew-point temperature. Thus at the lower end of the scale a 5° F. differential between −95° and −100° F. is equivalent to a change in the moisture content of 0.0002%, while in the 0.01% range a 5° F. change is equivalent to 0.0027% of moisture.

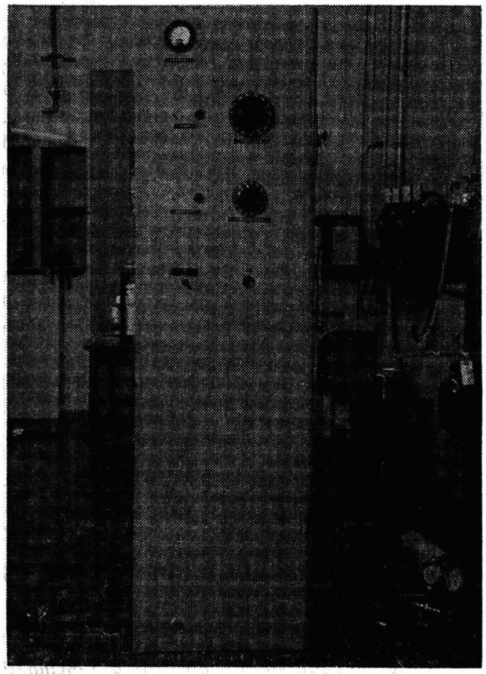

Figure 4. Left-hand view of oxygen analyzer

As a final test to determine the effectiveness of the instrument for quantitative measurement and incidentally to confirm that the observed responses were due only to oxygen and moisture, an estimate was made of the oxygen concentrations in several test gases. This was done by determining the straight dew point of the gas and the dew point of the gas when hydrogen is added. The oxygen value is the difference between these values expressed as volume per cent of moisture divided by two. The gases were then analyzed by the method of Pepkowitz and Shirley (*3*) to determine the oxygen values. The data obtained are given in Table I and indicate exceptionally good agreement between the two methods of analysis.

The data given in column 6 are individual values and are compared with the averages of two or three determinations listed in column 7. These data can thus be taken as an indication of the accuracy of the method.

The instrument is capable of exceptionally good precision. The data in Table II present two series of replicate measurements on nitrogen and helium at the lowest available oxygen concentrations. The data were taken in groups of three, spaced a week apart to avoid the reflection of any constant instrument error which may be present at any given time. The mean deviation is ±0.0001 volume %.

Table II. Replicate Data Obtained with Nitrogen and Helium

Nitrogen Containing 0.00083 Vol. % O_2[a]			Helium Containing 0.00113 Vol. % O_2[a]		
Dew point, ° F.	Dew point + H_2, ° F.	O_2, vol. %	Dew point, ° F.	Dew point + H_2, ° F.	O_2, vol. %
−60	−56	0.0008	−54	−50	0.0011
−60	−55	0.0011	−54	−49	0.0014
−60	−56	0.0008	−54	−50	0.0011
−56	−52	0.0010	−51	−47	0.0013
−59	−55	0.0009	−52	−49	0.0010
−61	−57	0.0008	−50	−47	0.0011
	Av.	0.0009		Av.	0.0012
	Mean dev.	±0.0001		Mean dev.	±0.0001

[a] Chemical analysis (*3*).

In high pressure gas cylinders, there is almost always stratification, which, if not recognized, will produce erroneous and discouraging results. This is almost always true of the first samples of gas removed. These replicate runs were made on gases from cylinders which were half emptied in the course of the preliminary experiments. In addition a number of cylinders contained free water and the moisture content of the gas increased as the cylinder was depleted; this should be taken into account if the moisture content as well as the oxygen content must be controlled or avoided.

The contribution in terms of total oxygen ($H_2O + O_2$) of the added hydrogen, is negligible. The hydrogen flow is 5 cc. per minute while the test gas flows at ~2800 cc. per minute. The total oxygen ($H_2O + O_2$) in the electrolytic hydrogen used was 0.04 volume %. Thus the positive error from this source is only 0.00007%. Because of this negligible effect, there is no need to use any special grade of hydrogen nor to resort to elaborate means of cleanup, such as diffusion through palladium. Stoichiometrically 5 cc. per minute of hydrogen are in excess of the usual oxygen concentrations in gases. This rate is 0.22 millimole per minute, which would be equivalent to 0.0892 volume % of oxygen in the test gas at a flow rate of 2.8 liters per minute.

There are no interfering compounds in the common gases such as nitrogen, argon, hydrogen, helium, or carbon dioxide. The only care required is to exclude particulate matter and oil vapors which may deposit on the mirror and necessitate cleaning. A simple glass wool filter in the gas supply will usually eliminate such interference. As the freezing point of carbon dioxide is −110° F., and it can be liquefied only under pressure, there is no interference from carbon dioxide.

ACKNOWLEDGMENT

The author is particularly indebted to William Moak of this laboratory for his valuable assistance in fabricating the prototype instrument and obtaining most of the data, and Florence Blinn of this laboratory for performing the chemical analyses for oxygen in the test gases.

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Received for review July 31, 1954. Accepted October 18, 1954. The Knolls Atomic Power Laboratory is operated by the General Electric Co. for the Atomic Energy Commission. Work carried out under Contract No. W-31-109 Eng-52.

Determination of Benzo[*a*]pyrene in Complex Mixtures

Use of Catalytic Iodination on Activated Alumina

RUSSEL TYE, MARY JANE GRAF, and A. WESLEY HORTON
Kettering Laboratory, University of Cincinnati, Cincinnati, Ohio

This paper describes the isolation and identification of the polycyclic hydrocarbon, benzo[*a*]pyrene, in a residual product of the catalytic cracking of petroleum, together with the analytical method developed for the estimation of the concentration of this compound in products of refining operations. A selected fraction of a given sample is obtained by the use of a standardized chromatography. Two equal portions of the fraction are taken, and one of them is subjected to a catalytic iodination on a column of activated alumina. Spectrophotometric measurement of the difference in absorbance between the iodinated portion and its mate is then used to determine the concentration of benzo[*a*]pyrene present.

A HIGHLIGHT of the many years of effort by numerous investigators to identify the carcinogenic constituents in certain tars produced from coal was the isolation and synthetic proof of structure of benzo[*a*]pyrene by Hieger (*10*) and Cook and Hewett (*5*). Although there has been no direct proof of the relationship of the pure hydrocarbon to human cancer, such effects on certain experimental animals have been well documented (*9*).

In the course of the current investigation of the possible carcinogenic properties of high boiling products from petroleum refining operations, it was observed that the ultraviolet absorption spectrum of a distilled fraction of a catalytically cracked residuum showed maxima indicative of the presence of benzopyrene in significant concentration. The compound was isolated by the following procedure in a sufficient state of purity for positive identification. As a matter of conservation of time, no attempt was made at quantitative separation.

Fourteen hundred grams of the oil were subjected to a simple vacuum distillation. A fraction, boiling at 205° to 275° C. at 0.5 mm. of mercury and weighing 175 grams, was subjected to chromatography on alumina, and fractions enriched in benzopyrene were selected spectrophotometrically. The chromatographic procedure was repeated five times, resulting in 6.5 grams of a red semisolid, the ultraviolet absorption spectrum of which indicated a content of benzopyrene between 0.8 and 2.5 grams (curve I, Figure 1). The formation of iodine complexes (*4*, *14*) followed by filtration was used to remove perylene and other unidentified compounds, reducing the weight of the concentrate to 5 grams. This was dissolved in benzene and extracted with cold, concentrated sulfuric acid. The acid was diluted with ice, and the resulting precipitate was dissolved in benzene (for spectrum, see curve II, Figure 1). Further fractionation by chromatography and fractional crystallization from *n*-heptane produced 0.116 gram of crude benzopyrene, having substantially the same ultraviolet absorption as that of an authentic sample.

Further recrystallizations from *n*-heptane and from ethyl alcohol produced 12 mg. of crystals, melting point 175–176.5° C. (corrected). The melting point of a mixture with synthetic benzopyrene was 176–177° C. (corrected), as compared with that of the pure material, 178–178.5° C. (corrected). The spectrum of the isolated compound is shown as curve III, Figure 1.

The method of Goulden and Tipler (*8*), as adapted by Waller (*17*), for the determination of benzopyrene in soot and city dusts utilizes chromatography and fluorescence spectroscopy, the final estimation of concentration being made by visual comparison of the spectrum of the unknown with that of a known sample composed of benzopyrene and suitable impurities. Falk *et al.* (*6*) and Wedgwood and Cooper (*18*) have used chromatography and ultraviolet absorption spectrophotometry to separate and identify polynuclear aromatic hydrocarbons from complex mixtures. Fieser and Campbell (*7*) have suggested that a more selective analytical method for benzopyrene might be developed if the hydrocarbon were first converted to the highly colored derivative resulting from its reaction with *p*-nitrobenzenediazonium chloride.

The method described in this paper attempts to utilize the chromatographic technique not only to concentrate the benzopyrene but to aid in identifying it by its rate of movement along a carefully standardized column. Secondly, a selection is made by catalytic iodination, and finally, identification and measurement are accomplished on the basis of the change in the ultraviolet absorption spectrum caused by the iodination. The use of difference spectra, such as that of Figure 2, furnishes a double qualitative confirmation that benzopyrene was actually the compound iodinated, as the curve not only shows the maximum at 405 mμ due to the derivative, but also negative peaks or inflections at approximately 368 and 389 mμ due to the hydrocarbon. The method is relatively fast, requiring about 3 hours per analysis, or less if multiple analyses are made simultaneously.

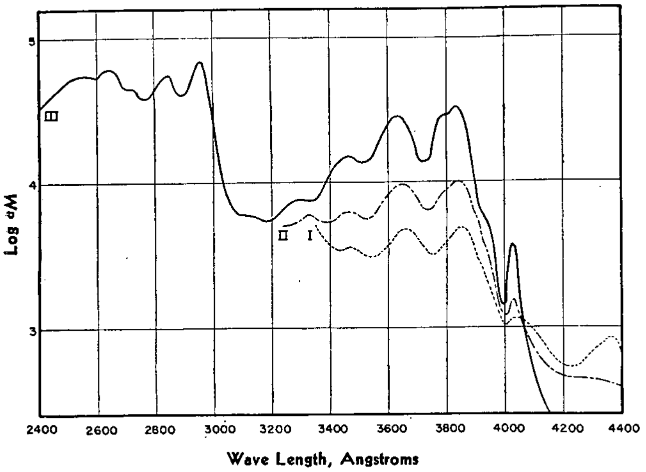


Figure 1. Ultraviolet absorption spectra of three stages in isolation of benzo[*a*]pyrene

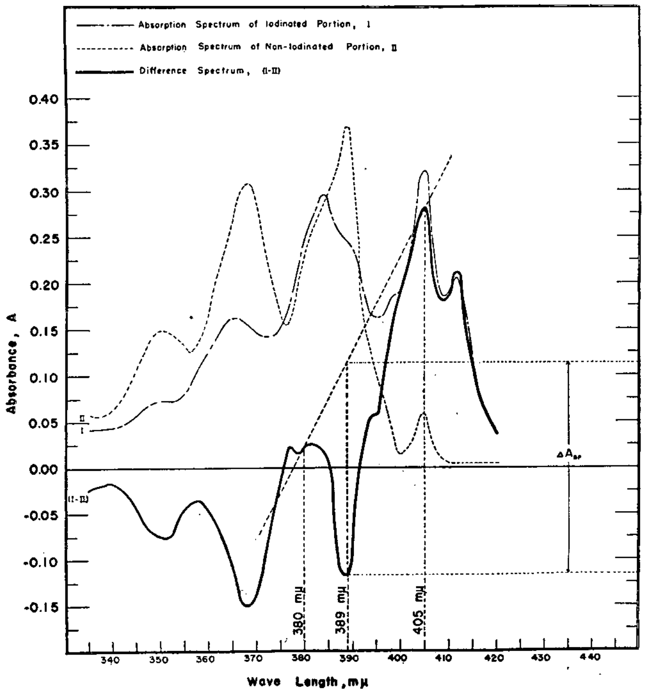


Figure 2. Spectra involved in analysis for benzo[*a*]pyrene

CATALYTIC IODINATION

In the case of certain severely cracked materials, such as some tars from by-product coke ovens, it is possible to obtain a reasonable estimate of the content of benzopyrene from the absorption spectrum of a suitably selected chromatographic fraction. However, for the majority of the mixtures examined by the authors, a considerable improvement in accuracy is obtained by converting the benzopyrene to a monoiodo derivative before spectrophotometric analysis. Any interference due to compounds which do not react with iodine under the experimental conditions may then be essentially eliminated by use of the technique of difference spectra for analysis of the derivative.

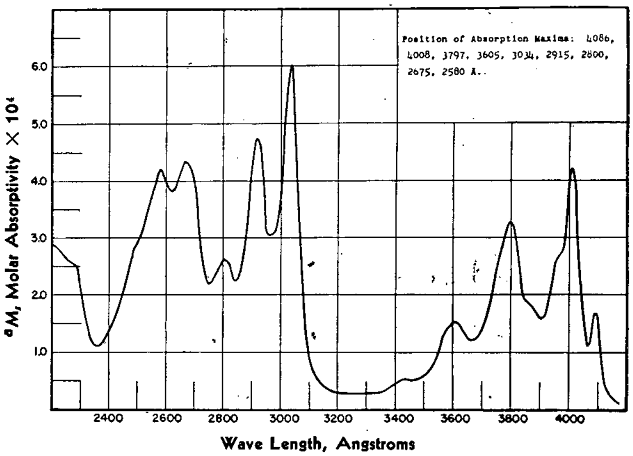
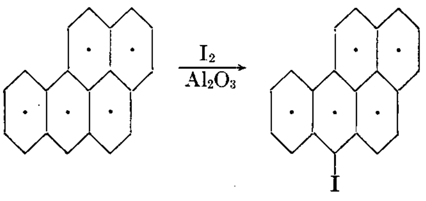


Figure 3. Ultraviolet absorption spectrum of 6-iodobenzo[*a*]pyrene in iso-octane

A convenient method of accomplishing the desired reaction is to pass a solution of benzopyrene and iodine in suitable concentration in benzene through a short column of activated alumina in the manner of elution chromatography. Under the conditions of the procedure used in this analysis, 6-iodobenzopyrene is produced in about 65% yield. (In the formulas below, the authors prefer the use of the symbolic π electron in presenting a resonance hybrid such as benzopyrene.)



Benzo[*a*]pyrene 6-Iodobenzo[*a*]pyrene

The iodo compound has been purified by chromatography and recrystallization. Its melting point was 214° to 215° C. (corrected); its iodine content (calculated as 33.6% for $C_{20}H_{11}I$) was 33.1%. The ultraviolet absorption spectrum is shown in

Figure 3; it is noteworthy that the compound does not have any visibly perceptible fluorescence.

The position of substitution was indicated by conversion of the iodo derivative to the nitrile by heating with a small excess of copper(I) cyanide [$Cu_2(CN)_2$] in a sealed glass tube at 306° C. for 1.5 hours. A yield of 97% of crude product was obtained. This product, when purified, melted at 239.0–239.5° C. (corrected). 6-Cyanobenzo[*a*]pyrene, prepared by the method of Windaus and Raichle (*19*), melted at 238–239° C. (corrected), and, in a mixture with the first cyano compound, melted at 238.5–239.0° C. (corrected). The ultraviolet spectra of the two samples were in agreement and corresponded with the spectrum obtained by Jones (*11*).

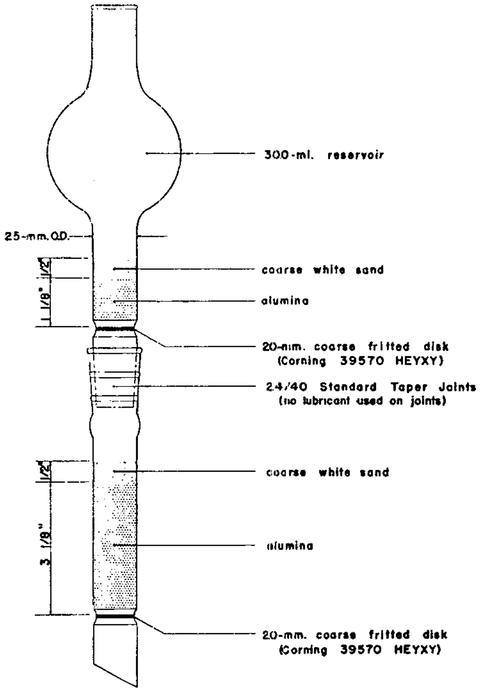


Figure 4. Apparatus

Other activated adsorbents also catalyze the iodination of benzopyrene. The pH of the adsorbent appears to be important. Under conditions similar to those of the analysis, the use of Attapulgus fuller's earth of low activity and of a lower pH than grade F-20 alumina (as measured by pH meter on aqueous extracts) produces roughly equal yields of 6-iodobenzopyrene and an additional product, probably an iodo- or diiodobenzopyrene. A similar mixture of products is obtained from the alumina catalyzed reaction if the acidity is raised by a large increase in the relative quantity of iodine used. Activated magnesia, having a pH much higher than grade F-20 alumina, and activated silica gel, having a pH much lower than the Attapulgus fuller's earth, promote only faint reactions. The use of silica gel to which sodium bicarbonate has been added results in an increased but still small amount of iodination. A mixture of sodium bicarbonate with celite shows no activity.

The relative ability of different aromatic hydrocarbons to form molecular complexes with Lewis acids has been discussed by several authors (*1–3*, *12*, *13*) and related by Mulliken (*13*) and Brown (*3*) to the ease with which these compounds may be halogenated. Brown found a simple linear relationship between the logarithms of the relative rates of halogenation of several methylbenzenes and the logarithms of the relative stabilities of their deeply colored onium-ion complexes with hydrogen fluoride-boron trifluoride. In dealing with mixtures of polycyclic aromatic hydrocarbons the difference between the basicity of the highly fused structures, such as benzopyrene, and that of other components seems to be the important characteristic which makes it possible in this analysis to eliminate many potential interferences by the iodination step.

The solubility of various polycyclic aromatics in cold, concentrated sulfuric acid (*4*) without sulfonation is presumably a function of the basic properties of the hydrocarbons. These solutions have vivid colors ranging from yellow to wine-red, suggesting the formation of onium-ion complexes (*3*, *13*). Perylene, 3-methylcholanthrene, benzopyrene, pyrene, and anthracene can be extracted from benzene with varying degrees of completeness by an equal volume of concentrated sulfuric acid at 10° C. Under the conditions of the analytical procedure, the same compounds react with iodine to similar relative extents. Benz[*a*]anthracene can be extracted by sulfuric acid but reacts negligibly with iodine. Chrysene, benzo[*c*]phenanthrene, dibenz[*a,h*]anthracene, and phenanthrene cannot be extracted by cold concentrated sulfuric acid, and are not iodinated by the analytical procedure.

A more generalized check on the apparent rule that polycyclic aromatic hydrocarbons not soluble in cold, concentrated sulfuric acid are not substituted by iodine under the conditions of this analytical method, has been made. The cracked residuum from which benzopyrene was isolated, believed to contain also a wide variety of other polycyclic aromatics including alkylated and hydrogenated derivatives, was exhaustively extracted by cold concentrated sulfuric acid, about 50% of the aromatic compounds being thus removed. Two equal portions of the nonextract (raffinate) were taken, one portion iodinated, and the difference spectrum measured according to the standard analytical procedure. No apparent reaction had occurred.

APPARATUS

The chromatographic column which is used to prepare a suitable fraction for the analysis is composed essentially of a reservoir and two separate sections of adsorbent, as shown in Figure 4. The spectrophotometer employed in the development of the method was the Beckman Model DU equipped with 1-cm. cells and a tungsten lamp. It is assumed that any comparable instrument, including the recording models, could be used equally well.

MATERIALS

Iso-octane, Phillips Petroleum Co., pure grade, redistilled.

Benzene, C.P. thiophene-free, redistilled.

Solution of benzo[*a*]pyrene, Edcan Laboratories, C.P., 5.00 mg. in 100.0 ml. of benzene.

Developing solvent, mixture of 200 ml. of iso-octane (2,2,4-trimethylpentane) with 200 ml. of benzene.

Solution of iodine (reagent grade), 1 gram in 10 ml. of benzene, freshly prepared.

Activated alumina, Alcoa, Grade F-20, 80 to 200 mesh.

Solution of sodium thiosulfate, C.P., approximately 5 grams, with approximately 50 mg. of potassium iodide, C.P., in 200 ml. of distilled water.

Sand, nonporous, white silica.

PROCEDURE

Volume of Developing Solvent Required for Satisfactory Chromatographic Fractionation. A chromatographic column of alumina is prepared as shown in Figure 4. Because the quantity of adsorbent used is measured volumetrically in the column itself, it is important that the procedure for packing the columns in this and the next step of the procedure be as uniform as possible. A satisfactory routine consists of tapping the loosely filled column six times with a pencil. Ten milliliters of the developing solvent are poured on to the column followed by 10 ± 0.05 ml. of the solution of benzopyrene and then by an additional 100 ml. of the solvent. Each portion of liquid should be allowed to enter the top layer of sand before the next is added. Additional solvent is then added, 10 ml. at a time, until the top of the benzopyrene band, as indicated by blue-violet fluorescence under ultraviolet light (the General Electric Co. Purple-X lamp bulb is a satisfactory source of light for this purpose), is approximately 0.25 inch below the top of the lower section of alumina. At this point, the fluorescent compound will usually be spread over about one third of the lower section of alumina.

With adsorbent of normal activity, this development will require a total volume of about 120 to 150 ml. of the solvent (not including the two 10-ml. portions used to prewet the column and as solvent for the benzopyrene). The total volume of solvent thus determined is used as the "standard volume" for the separatory chromatography (below). The use of alumina requiring a "standard volume" of solvent less than 100 ml. is to be avoided if possible, as the effectiveness of the separation of benzopyrene from interfering components is thereby reduced. The only known disadvantage in the use of alumina requiring a standard volume greater than 170 ml. is the additional cost in time and material. Alumina of unusually high or low activity could probably be used satisfactorily by modification of the composition of the developing solvent, but should in that case be checked further with pure benzopyrene to determine whether it has a satisfactory catalytic action in the iodination reaction described below.

As a rule, the activated adsorbent needs to be calibrated only once for a given series of analyses, so long as all of the alumina is taken from the same can and adequate care is taken to minimize the periods during which the can is open and to keep it tightly sealed at other times.

Preparation of Fraction for Iodination. Approximately 100 mg. (weighed to the nearest 0.1 mg.) of the material to be analyzed are dissolved in 5 ml. of benzene. After the material is completely in solution, 5 ml. of iso-octane are added. (If part of the material is insoluble in benzene, or if precipitation occurs on addition of iso-octane, the sample should be subjected to a special procedure. See modification below.) The material is then fractionated on a freshly packed chromatographic column in the exact manner described above, substituting this solution of the unknown for the solution of benzopyrene used to calibrate the alumina.

After the standard volume of the developing solvent has run through the column, the sections are separated and the material adsorbed on the lower section is eluted by 50 ml. of a solution of 20% ethyl alcohol and 80% benzene (by volume). Most of the solvent is removed from the eluate by evaporation on a steam bath. The remainder is removed by blowing under a stream of nitrogen with care to avoid loss by spattering. The residue, representing a concentrate of any benzopyrene present in the sample, is then ready for the iodination step.

MODIFICATION. The suspension of 100 mg. of the sample in either 5 ml. of benzene or 10 ml. of the 50 to 50 mixture of benzene and iso-octane, as the case may be, is poured onto a column of alumina 1 inch in diameter and 1 inch deep (comparable to the upper section of the column in Figure 4). The flask in which the suspension was prepared is washed thoroughly with 10-ml. portions of benzene, and the washings are added to the column. After this material has passed into the adsorbent, the column is eluted with 100 ml. of benzene. Most of the solvent is removed from the eluate by evaporation on a steam bath, the remainder by careful blowing under a stream of nitrogen. The residue obtained is dissolved in 5 ml. of benzene, 5 ml. of iso-octane are added, and the analysis resumed at the point of interruption.

Iodination. The residue from the step above is dissolved in 15 ± 0.05 ml. of benzene, and two 5-ml. portions, labeled I and II, of the resulting solution, are transferred to two clean 125-ml. flasks, using the same pipet for each. Five milliliters of the solution of iodine are added to I, and 5 ml. of benzene added to II. Two parallel columns of alumina are prepared, each 1 inch deep and 1 inch in diameter, covered with 0.5 inch of sand. Each column of adsorbent is wet by 15 ml. of benzene and allowed to drain until the frequency of drops is less than one in 10 seconds. Clean, tared, 125-ml. flasks are then placed under the columns. Five milliliters of the solution of iodine, diluted with 5 ml. of benzene, are poured onto the first column, and 10 ml. of benzene alone are poured onto the second. Solutions I and II are then added to the respective columns, the flasks which had contained them are rinsed by 10 ml. of benzene, and the rinsings added to the proper columns. Each is then eluted by 70 ml. of benzene and allowed to drain as before.

The eluate from column I, containing the iodine, is then shaken with 200 ml. of the aqueous sodium thiosulfate in a 500-ml. separatory funnel, after which it is washed with two 100-ml. portions of distilled water. The eluate obtained from column II is used directly in the final step without further handling.

The processed fractions, I and II, should be subjected to the spectrophotometric analysis without delay, since the iodinated sample occasionally proves to be unstable on standing overnight.

Determination and Analysis of Difference Spectrum. The algebraic differences between the absorbances of the final fractions, I and II, in the 370 to 420 mμ range are now measured directly by use of a Beckman DU spectrophotometer. Sample I is placed in the 1-cm. cell normally used for solutions, and sample II in the 1-cm. cell used for blanks. Obviously, the cells used must be well matched as to length. The instrument is operated in the normal manner except that, when II has the higher absorbance, the machine is balanced on I, and the absorbance is recorded as a negative value (see Figure 2). The slit width must be kept moderately low, below 0.1 mm. being desirable. The sensitivity knob should therefore be turned to a point near its extreme counterclockwise position (minimum sensitivity), and the tungsten lamp should be used. The reduced slit widths are necessary because the iodinated solution I usually has a markedly lower level of fluorescence than the solution II. This difference assumes critical importance when compared with the difference in absorption rather than with the usual total absorption. Hence, if the slit width required at 380 mμ exceeds 0.15 mm., 10.00 ml. of each solution should be diluted with 50.00 ml. of benzene prior to spectral analysis.

Satisfactory analyses may be obtained from difference spectra in which the measured absorbance at 389 or 405 mμ is 0.02, or greater, so long as each point is determined carefully and the absorbance is plotted on a suitable scale. If the absorbance is less than 0.02, longer cells (5- or 10-cm.) should be used. Alternatively, solutions I and II may be concentrated by the following procedure: The weights of the solutions are determined. Then they are evaporated on a steam bath sufficiently to obtain the desired level of absorbance. Their reduced weights are adjusted to equal percentages of their respective original weights with benzene (this percentage is then used as the value of the factor, V, in the equation below).

The difference in absorbance is determined at 380, 389, and 405 mμ and at every interval of 4 mμ in the region 420 to 370 mμ. The following base-line technique (*15, 20*) is applied to the difference spectrum obtained: A line is drawn intersecting the curve at the points corresponding to 405 and 380 mμ. The distance (in absorbance units) along the vertical coordinate at 389 mμ from the curve to this line is designated as ΔA_{BP} (see Figure 2). Geometrical analysis of the system provides the following relationship:

$$\Delta A_{BP} = 0.64A_{380} + 0.36A_{405} - A_{389}$$

Direct use of this equation without plotting is warranted only when experience has provided satisfactory qualitative evidence of the presence of benzopyrene and absence of significant interference from perylene.

The percentage of benzopyrene in the original sample is calculated from the equation:

$$\text{Per cent benzopyrene} = \frac{V}{W_s l} \times \frac{\Delta A_{BP}}{0.23}$$

where W_s = original weight of sample in milligrams

V = 100 if no dilution or concentration of the final solutions was required

V = 600 if dilution was required

$V = \frac{W_2}{W_1} \times 100$ if concentration was required

W_2 = the weight of the iodinated fraction after concentration

W_1 = the weight of the iodinated fraction before concentration

l = length of spectrophotometer cells in centimeters

The constant, 0.23, is the slope of the essentially linear relationship between ΔA_{BP} and the content of benzopyrene over the range of concentrations studied, 0.01 to 1.5%.

SENSITIVITY AND REPRODUCIBILITY

Consideration of various factors inherent in the procedure indicates that the limit of sensitivity of the method under ideal conditions is about 0.0001 mg. of benzopyrene. In actual analyses, the presence of other compounds, which modify the shape of the final difference spectra, limits the practical sensitivity to about 0.001 mg.

Quantities of pure benzopyrene sufficient to provide from 0.01 to 0.5% were dissolved in several different oils of widely varying type, to which this analytical procedure had been applied previously. In each case in which the amount of added benzopyrene represented a significant increase in the total present, the standard analysis yielded a reasonably precise measure of the quantity added, as shown by the examples in Table I.

Data from a number of analyses involving duplication by four different operators indicate a reproducibility within the limits of

Table I. Precision of Method

Oil Number	*A* Benzo[*a*]pyrene Found in Original Sample, %	*B* Concentration of Benzo[*a*]pyrene Added, %	*A* and *B* Indicated Total, %	Total Determined, by Analyses, %
1	0.015	0.101	0.116	0.114
2	0.428	0.490	0.918	0.909
3	0.0012	0.0100	0.0112	0.0111
4	(0.0002)	0.0099	0.0101	0.0098
5	0.086	0.198	0.284	0.283

about ±20% for samples with benzopyrene concentrations around 0.01%, ±10% for concentrations of 0.10%, and ±5% for concentrations of 0.50% or greater. Seven other research laboratories are now cooperating in a further evaluation of the analytical method.

INTERFERENCES

Many compounds other than benzopyrene react with iodine under the conditions of the iodination step of the analysis. Among these are anthracene, pyrene, 3-methylcholanthrene, and perylene. The majority of such compounds are separated from benzopyrene by the chromatographic fractionation step. Of the compounds which accompany benzopyrene through this separation and the subsequent iodination, the alkyl derivatives of benzopyrene are perhaps the most important potential sources of interference, if present in any significant concentration. It is not known how their presence would effect the final analysis. The following experiments were carried out in an effort to obtain some estimate of the extent of such interference, if any.

Analysis of a 20% residuum from distillation of the oil from which benzopyrene was isolated (No. 2 in Table I) indicated the presence of 1.8% of this hydrocarbon. A second sample of this residuum was fractionated according to the second step of the procedure. One fifth (termed fraction A in Table II) of the chromatographic fraction containing the benzopyrene was then subdivided by a more refined chromatography into three fractions containing approximately equal portions of benzopyrene, and these were analyzed by iodination and difference spectra. A measured quantity of synthetic benzopyrene was then added to a similar one-fifth portion of the standard chromatographic fraction from the oil. This sample was then subdivided by a careful reproduction of the previous chromatography, and the three resulting fractions were analyzed. Duplications were then made for both experiments. Finally, pure benzopyrene was processed chromatographically in duplicate experiments similar to the preceding ones, and the resulting fractions were analyzed. The results of these experiments are shown in Table II.

In cases of samples 1 and 2, percentages are based on the total benzopyrene indicated by summation of the weight of synthetic benzopyrene added and that determined by independent ordinary analysis of fraction A. The table shows that the apparent benzopyrene from the oil does not differ chromatographically from synthetic benzopyrene. There is some reason, therefore, to believe that alkylbenzopyrenes are creating no serious interference in the analysis of the oils.

Although perylene and some of its alkyl derivatives remain in the benzopyrene fraction obtained from chromatography, their concentration in cracked products is usually so low, relative to that of the benzopyrene, that it does not interfere significantly. In the analysis of certain straight-run distillates, however, the perylene family does create a positive error. A high, positive slope of the final difference spectrum in the 410 to 420 mμ range is an indication of such an interference, which may be confirmed by finding maxima due to iodoperylenes at 450 to 458 mμ and 422 to 425 mμ, and corresponding minima due to the hydrocarbons at approximately 438 and 410 mμ. The value of ΔA_{BP}, used to calculate the content of benzopyrene, should then be corrected by subtracting the quantity 0.1 ΔA_P where:

$$\Delta A_P = 0.5A_{422} - A_{438} + 0.5A_{451}$$

In the analysis of certain types of materials, such as crude residua, difference spectra are occasionally obtained which lack sharp maxima and minima or well defined inflections and do not furnish satisfactory qualitative evidence of the presence of benzopyrene. Hence, the significance of results obtained by application of the base-line technique to such spectra is uncertain. Various methods of eliminating the obscuring interference are being examined.

Table II. Chromatographic Comparison of Apparent Benzopyrene from a Complex Oil with Pure Compound

Sample	Total Benzo[*a*]pyrene Present, %			
	Fraction 1	Fraction 2	Fraction 3	Fraction 1 + 2 + 3
1. Fraction A of cracked residuum	29	39	31	99
	31	42	27	100
2. Fraction A + added benzopyrene	31	42	25	98
	34	41	22	97
3. Pure benzopyrene	31	47	20	98
	28	46	26	100

A technique has been devised which, applied to such materials, provides evidence that the actual concentration of benzopyrene is, at most, no higher than that indicated by the standard procedure. The values in question have usually been less than 0.003%. It has been found that a significant proportion of the compounds responsible for the obscuration of the difference spectra may be modified in composition (possibly polymerized) by subjection of a weighed quantity (approximately 100 mg.) of the sample to what amounts to a flash distillation at atmospheric pressure without reflux. Application of the standard method of analysis to the distillate then yields a much improved difference spectrum. In our experience, the value of the concentration of benzopyrene, thus determined, has not proved to be higher than that obtained by the routine method. Indeed, agreement between the values obtained by the two procedures has been rather striking.

APPLICABILITY

The method was developed primarily for application to various petroleum products resulting from refining operations. It has been applied to more than 35 such materials and the analytical data thus far obtained have been plausible in the light of the history and the physical properties of the samples. The use of the method has been extended, in a limited manner, to substances derived from other sources, including coal tars and fractions thereof. No apparent difficulties were encountered with these materials. It seems likely that the analysis may be applied to mixtures extracted by benzene from a variety of additional materials such as soot, carbon black, condensates from atmospheric samples, and other materials which are derived from fossil fuels or the incomplete combustion of organic mixtures. It should be recognized that the results of analyses of solid materials of limited solubility in benzene would not necessarily indicate the total amount of benzopyrene in the original sample, since the complete extraction of benzopyrene from such materials is frequently very difficult to achieve *(16)*.

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RECEIVED for review May 10, 1954. Accepted October 23, 1954. The majority of this work was carried out as part of the American Petroleum Institute Research Project MC-1.

Copper(I)-2,2′-biquinoline Complex in Aqueous Dimethylformamide

RONALD T. PFLAUM, ALEXANDER I. POPOV, and NEIL C. GOODSPEED

Department of Chemistry, State University of Iowa, Iowa City, Iowa

The copper(I)–2,2′-biquinoline complex was investigated in order to determine the nature of the absorbing species in a water-miscible solvent and the feasibility of utilizing this solvent in determining copper. The bis-2,2′-biquinoline-copper(I) ion was found to be responsible for the characteristic purple color developed in the reaction of the reagent with cuprous copper. A mixture of equal parts by volume of dimethylformamide and water was a satisfactory medium for this chelation reaction. Such a solvent mixture functions in the role of a reducing agent in the reduction of copper(II) to copper(I) ion. As a consequence, it was unnecessary to add a reductant to the system. Reducible ions and those that enter into precipitation reactions interfere in the development and measurement of color. The results obtained on the determination of copper in selected samples indicate the feasibility of employing the described system for such determinations. An unreported maximum in the absorption curve of the complex was found and described. The ultraviolet absorption curves for the reagent in dimethylformamide and in isoamyl alcohol were obtained.

THE color reaction between the organic base, 2,2′-biquinoline, and copper(I) ion was first observed by Breckenridge, Lewis, and Quick in 1939 (*1*). Since that time, several investigators have studied the colored system from the standpoint of the selectivity of the reaction (*3, 5, 6*). Breckenridge and coworkers (*1*) utilized glacial acetic acid as a solvent in their studies whereas Hoste and coworkers (*3, 5, 6*) have restricted themselves to the use of water-immiscible alcohols. These latter investigators have determined that the reagent is specific for copper and have proposed that a bis-2,2′-biquinoline–copper(I) species existed in these nonaqueous media.

The specificity of this reaction has led to some interesting proposals for the steric requirements of the reagent in the chelation process (*2*) and lends great analytical significance to its use. Applications of the colored complex to the determination of copper in a variety of samples have been described (*4, 7, 8*). All the determinations have been carried out in amyl or isoamyl alcohol with the use of hydroxylamine hydrochloride for the reduction of copper(II) to copper(I) ion. The standard analytical procedure requires a change in the oxidation state of the metal ion and an extraction of the colored species out of aqueous solution into a nonaqueous phase.

In view of the specificity of this cuproine reagent, it seemed desirable to carry out a rigorous determination of the formula of the colored species involved in the reaction. This investigation is concerned with such a study, together with the application of new conditions in the determination of copper. These permit the use of a simplified procedure in the analysis of certain selected samples.

APPARATUS AND REAGENTS

All spectrophotometric measurements were made with a Cary recording spectrophotometer. One-centimeter matched silica cells were used in every measurement. A Beckman Model G pH meter was used for all pH measurements.

The dimethylformamide was obtained from the Rohm and Haas Co. Purification was effected by treatment with barium oxide for a period of 24 hours with subsequent rectification in an all-glass system. The fraction boiling at 151° ± 1° C. was utilized as the purified solvent. The 2,2′-biquinoline, obtained from the G. Frederick Smith Chemical Co., was used as received.

A standard solution of copper(II) ion was prepared by dissolution of pure copper wire in nitric acid and conductivity water. The pH of the final solution was adjusted to 5 with sodium hydroxide.

All other chemicals used were of reagent grade quality.

EXPERIMENTAL

Investigation of Reagent. An investigation of the reagent was undertaken initially in order to ascertain its solubility and absorption characteristics. It was found that 2,2′-biquinoline is sparingly soluble in ethyl alcohol, dioxane, acetonitrile, and isoamyl alcohol, but very soluble in dimethylformamide. Dissolution in dimethylformamide is not accompanied by any color change nor is color developed on standing for at least 2 months. The reagent easily retains its chelative powers for this period of time. Thus, dimethylformamide appears to be an ideal solvent for 2,2′-biquinoline.

Absorption curves for 2,2′-biquinoline in the ultraviolet region of the spectrum are shown in Figure 1. Curves for the reagent in isoamyl alcohol and in dimethylformamide are presented. The same absorption characteristics were observed in both solvents. No variations were apparent for fresh and aged solutions. Recrystallization of the reagent from aqueous alcoholic mixtures did not result in changes in the absorption curve.

Effect of Solvent on Color Reaction. The addition of solid 2,2′-biquinoline to a dimethylformamide solution containing $1 \times 10^{-3}M$ copper(II) ion produced a faint purple color. The subsequent addition of 0.05 gram of hydroxylamine hydrochloride completely removed any purple color formed, and resulted in a colorless solution. The addition of water to a solution containing the reagent and copper(II) ion in dimethylformamide or to a dimethylformamide solution containing the reagent, copper ion, and hydroxylamine hydrochloride resulted in the formation of an intense purple color. The maximum color in the reaction was developed in the absence of hydroxylamine hydrochloride or a similar reductant. Thus, the addition of a reducing agent to an aqueous dimethylformamide mixture is unnecessary.

The observations described above point to an interdependence of water and the organic solvent and to the function of such a mixture as a reductant in the reduction of copper(II) to copper(I) ion in the presence of 2,2′-biquinoline. It was found that maximum color developed in solutions containing a definite proportion of water. Solutions containing 40 to 60% of water by volume showed maximum color formation. Color in these systems was stable for at least 3 weeks. As a consequence, studies were carried out in 50% dimethylformamide with the solvent acting in a dual role—i.e., as the reductant and as a medium for the chelation reaction.

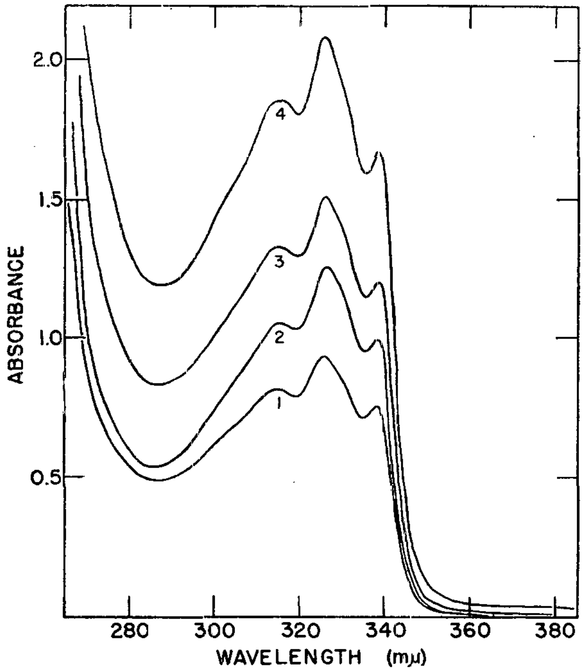

Figure 1. Absorption spectra of 2,2′-biquinoline

1. $4.25 \times 10^{-5}M$ 2,2′-biquinoline (recrystallized) in dimethylformamide
2. $5.70 \times 10^{-5}M$ 2,2′-biquinoline in isoamyl alcohol
3. $7.0 \times 10^{-5}M$ 2,2′-biquinoline in dimethylformamide (5 weeks old)
4. $1.0 \times 10^{-4}M$ 2,2′-biquinoline in dimethylformamide (fresh)

Effect of Reagent Concentration. A continuous variations study was carried out according to the method of Job (*9*) in order to determine the formula of the complex and the effect of reagent concentration. Stock solutions of $1 \times 10^{-3}M$ copper(II) ion (aqueous) and $1 \times 10^{-3}M$ 2,2′-biquinoline (in dimethylformamide) were used for this purpose. Solutions containing varying ratios of reactants were prepared in the usual manner. The resulting mixtures of 10-ml. volume were diluted to 25 ml. with water and dimethylformamide, so that final solutions contained 50% by volume of dimethylformamide. Absorbance measurements were made at 500, 545, and 575 mμ for the series of eight solutions.

Continuous variations plots at 500 and 545 mμ are shown in Figure 2. Corrected absorbance values are plotted against the ratio of reagent to copper ion. It is readily apparent that the characteristic purple color in the reaction is due to a 2 to 1 absorbing species. The sharpness of the maxima indicates a high degree of stability in the complex. It is therefore unnecessary to have excess reagent (greater than a 2 to 1 ratio of reagent to copper ion) for maximum color formation. The absorbance values obtained in this study show that excess reagent does not interfere in color development or measurement.

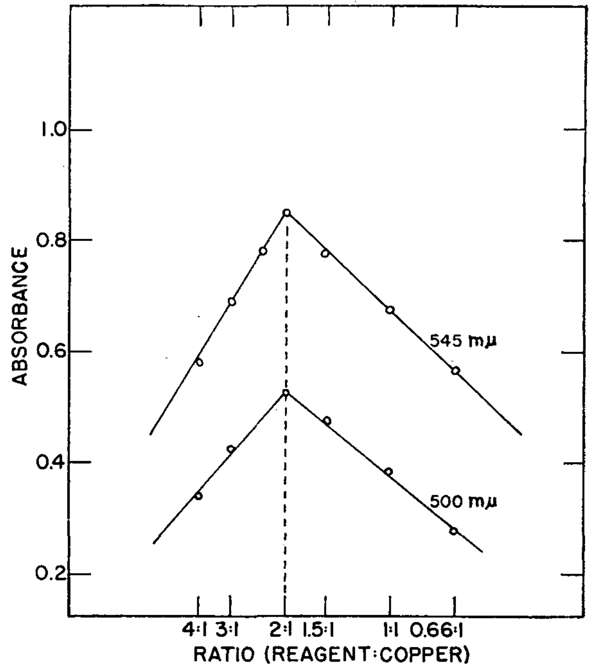

Figure 2. Continuous variations plots for copper–2,2′-biquinoline system in 50% aqueous dimethylformamide

Effect of Copper Concentration. Solutions containing varying concentrations of copper and the reagent were prepared and measured in order to determine the conformance to Beer's law and to make calculations of the molar absorptivity of the complex. Solutions containing $1.50 \times 10^{-5} - 3.50 \times 10^{-4}M$ copper(II) ion and a threefold concentration of reagent were studied. The absorption curves for four such aqueous dimethylformamide solutions are shown in Figure 3.

Absorption maxima occur at 358 and 545 mμ. Molar absorptivities, based upon concentration of copper(II) ion in moles per liter, are 52,000 and 6450 respectively. The former value is approximate inasmuch as excess reagent masks the absorption maximum. This maximum has been unreported heretofore. The great sensitivity exhibited shows promise for the utilization of the reagent in detecting micro amounts of copper. At 545 mμ, the system conforms to Beer's law in the concentration range $1.72 \times 10^{-5} - 2.75 \times 10^{-4}M$ (1.1 to 17.4 p.p.m. of copper).

Effect of pH. The sensitivity of the system to conditions of acidity was overcome by controlling the pH of the standard copper solution or of the solution containing the copper sample. The adjustment of pH was made by the addition of dilute sodium hydroxide to these aqueous solutions. With the pH adjusted to 5.0 to 7.0, the color of the complex in the resulting 50% aqueous dimethylformamide medium developed satisfactorily. Reproducible results were obtained when the pH of the aqueous solution was adjusted to 5.0 to 6.0.

Effect of Diverse Ions. Although the color reaction is specific for copper, interferences due to certain diverse ions were encountered in the system described. These occur in the presence of colored metallic ions and ions that either cause precipitation reactions or introduce undesirable oxidative effects. Advantage of specificity was taken in the study of interferences such that the particular ion in question was added to the blank and to the colored chelate solution. Chloride and nitrate salts, readily soluble in 50% aqueous dimethylformamide, were added in appropriate amounts to solutions containing 13 p.p.m. ($2.04 \times 10^{-4}M$) copper(II) ion. Interferences in the clear solutions were determined spectrophotometrically.

A list of the common ions studied is presented in Table I. The effects of the colored metal ions were cancelled out by use of a proper blank solution. Silver ion is partially reduced to metallic silver and is precipitated as such. Aluminum, iron, and lead may interfere through precipitation. Lead can be separated in the preparation of the sample and the addition of tartaric acid will prevent the precipitation of aluminum and iron. Ferric ion and similar oxidants interfered in the development of color. Addition of masking agents such as tartrate, citrate, and fluoride did not prove entirely satisfactory in eliminating the undesirable oxidative effects of these ions. Relatively large amounts of iron can be present in the lower oxidation state. The addition of a reductant, hydroxylamine hydrochloride, eliminated the interferences due to iron.

Table I. Effect of Diverse Ions

Ion	Permissible Amt., P.P.M.	Ion	Permissible Amt., P.P.M.
$C_2H_3O_2^-$	2000	Mg^{++}	500
Al^{+++}	800	Mn^{++}	500
Cd^{++}	500	Ni^{++}	500
Cl^-	2000	NO_3^-	2000
Cr^{+++}	500	ClO_4^-	2000
Co^{++}	500	K^+	2000
F^-	300	Ag^+	0
Fe^{++}	300	Na^+	2000
Fe^{+++}	10	SO_4^{--}	1000
Pb^{++}	10	Zn^{++}	500

SUGGESTED METHOD FOR DETERMINING COPPER

Add 20 ml. of 6N hydrochloric acid to 0.5 gram of the sample in a 250-ml. beaker, and heat to boiling. Cool, add 5 ml. each of concentrated nitric and sulfuric acids, and evaporate to fumes of sulfuric acid. After cooling, add 30 ml. of water, boil to dissolve the soluble salts, and filter off the insoluble sulfates and siliceous material. Wash the residue with hot water and dilute the filtrate to an appropriate volume in a volumetric flask. Add 1 gram of tartaric acid to the solution and adjust the pH to 5.0 to 6.0 with dilute sodium hydroxide.

Add 5 ml. of the aqueous copper solution to 12.5 ml. of 1 × $10^{-3}M$ reagent in dimethylformamide.

Dilute the mixture to 25 ml. with distilled water.

Prepare a blank by adding 5 ml. of the sample solution to 12.5 ml. of dimethylformamide and diluting to 25 ml. with water.

Measure absorbance at 545 mμ spectrophotometrically. Determine copper concentration from the absorbance value and a prepared calibration curve.

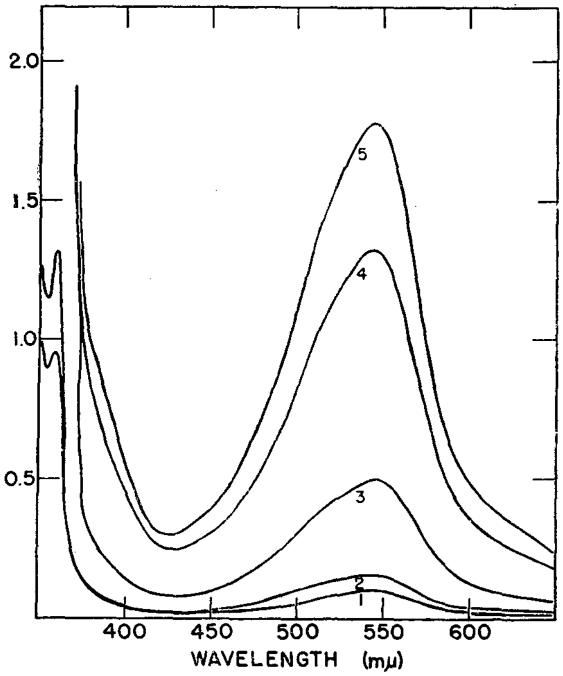

Figure 3. Absorption curves for copper(I)–2,2′-biquinoline complex in 50% aqueous dimethylformamide

1. 1.72 × $10^{-5}M$ copper
2. 2.5 × $10^{-5}M$ copper (isoamyl alcohol)
3. 7.02 × $10^{-5}M$ copper
4. 2.02 × $10^{-4}M$ copper
5. 2.75 × $10^{-4}M$ copper

Table II. Results of Determinations

Sample	Type	Copper Present, %	Copper Found, %
NBS 67	Manganese metal	0.16	0.17
NBS 86	Aluminum alloy	7.66	7.64
S-51	Co, Ni, Cu	0.25	0.25
S-101	Co, Ni, Fe, Cu	1.25	1.23
S-151	Zn, Cd, Mg, Al, Cu	0.44	0.45
S-202	Cr, Mn, Cu	0.67	0.63

RESULTS OF DETERMINATIONS ON SELECTED SAMPLE

The results obtained with this method are shown in Table II. Synthetic samples were prepared from a mixture of soluble nitrate and perchlorate salts. National Bureau of Standards samples were chosen for very low concentrations of iron. Results indicate that the method is applicable to determination of copper in samples low in iron content.

CONCLUSIONS

The results of this investigation prove that the specific reaction between copper ion and 2,2′-biquinoline can be carried out and studied in a new solvent medium. The use of this solvent, 50% aqueous dimethylformamide, eliminates the necessity for reduction and extraction in the preparation of the colored complex. The color of the reaction was found to be due to the bis-2,2′-biquinoline–copper(I) ion. Absorption spectra of this chelate in the dimethylformamide medium show maxima at 358 and 545 mμ exhibiting molar absorptivities of 52,000 and 6450, respectively. Absorption spectra of the reagent in the pure solvent and in isoamyl alcohol show maxima at 315, 326, and 338 mμ.

The feasibility of employing 2,2′-biquinoline in dimethylformamide for the determination of copper was demonstrated. The system described obeys Beer's law, is independent of reagent concentration, and exhibits a very high degree of stability. The method proposed is sensitive and is noteworthy for its simplicity. The results on several samples show that accurate determinations can be obtained.

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Received for review July 23, 1954. Accepted October 25, 1954. Presented before the Division of Analytical Chemistry at the 125th Meeting of the American Chemical Society, Kansas City, Mo.

Colorimetric Determination of Manganese

Oxidation with Bromate in Sulfuric Acid Medium

WILLIAM C. PURDY and DAVID N. HUME

Department of Chemistry and Laboratory of Nuclear Science, Massachusetts Institute of Technology, Cambridge 39, Mass.

A method has been developed for the colorimetric determination of manganese in the centigram range based on the oxidation of manganous ion to a trivalent sulfate complex with bromate in 8*M* sulfuric acid solution. The bromine color is discharged with cyanide ion. The complex, measured at 500 mμ, obeys Beer's law over the range of 2 to 70 mg. of manganese per 100 ml. of solution, and the color is stable for one week. Interfering substances are reducing agents, chromic ions, and ions forming insoluble sulfates. The effect of the latter can be removed by filtration.

THE present investigation was the result of a need for a simple colorimetric method for measuring the recovery of added manganese carrier in the determination of trace manganese through radioactivation. One characteristic desired for the method was that it be applicable to samples containing 10 to 50 mg. of manganese without extensive dilution or subsampling. The high sensitivity of the conventional colorimetric determination of manganese by oxidation to permanganate, although highly desirable with samples in the microgram range, becomes more inconvenient when centigram amounts of manganese are involved. Alternative methods were considered, and the method of Tomula and Aho (*5, 6*) was investigated because it appeared to be a simple procedure with satisfactorily low sensitivity and relatively few interferences. In it, manganous ion in a sulfuric acid solution was oxidized with bromate to the tripositive state which was stabilized by complexation with "sirupy pyrophosphoric acid." According to the authors, the complex had an absorption peak at 500 mμ and obeyed Beer's law from 2 to 40 mg. of manganese per 100 ml. of solution. In attempting to study the reproducibility and sensitivity of their method, the authors were unable to obtain consistent results. However, it was found that manganese could be determined satisfactorily as the trivalent sulfate complex in strong sulfuric acid.

EXPERIMENTAL

When the procedure of Tomula and Aho was followed with the substitution of tetrapotassium pyrophosphate for sirupy pyrophosphoric acid, no color was obtained. Only when the acid concentration was increased 2.5-fold did a color appear The reproducibility under these conditions was very poor, with duplicate samples often differing by 10%.

A factorial experiment was run in order to determine the factors affecting complex formation. These factors, each at two levels, were acidity, concentration of manganese, concentration of pyrophosphate, and the temperature of the reagents at the time of color development. The results of this experiment indicated that acidity was an important positive factor and that there was a strong positive interaction between acidity and manganese concentration.

The effect of using different phosphates in place of pyrophosphate was then investigated: orthophosphate from orthophosphoric acid, metaphosphate from orthophosphoric acid heated until the appearance of fumes, polymetaphosphates from Calgon, pyrophosphate from tetrapotassium pyrophosphate (City Chemical Co.) and from orthophosphoric acid heated at 200° C. for 1 hour, and tripolyphosphate from sodium tripolyphosphate (Monsanto Chemical Co.). All of the experiments were performed at the same acidity, 2.7*M* hydrogen ion, with several concentrations of manganese. The colors produced varied from amber to magenta. The spectra, together with the curve reported by Tomula and Aho, are shown in Figure 1. Distilled water was used as the blank. Sodium polymetaphosphate (Calgon), pyrophosphoric acid, metaphosphoric acid, and sodium dihydrogen pyrophosphate (Monsanto Chemical Co.), or solutions of pyrophosphate which had stood for some time at about pH 3, gave essentially the same curves. Different absorption curves were obtained, however, with orthophosphoric acid, tetrapotassium pyrophosphate, and sodium tripolyphosphate.

Since a colored complex could be obtained using orthophosphate instead of pyrophosphate, this system was investigated by a factorial experiment. The factors were the same as previously used, except for the substitution of orthophosphate for

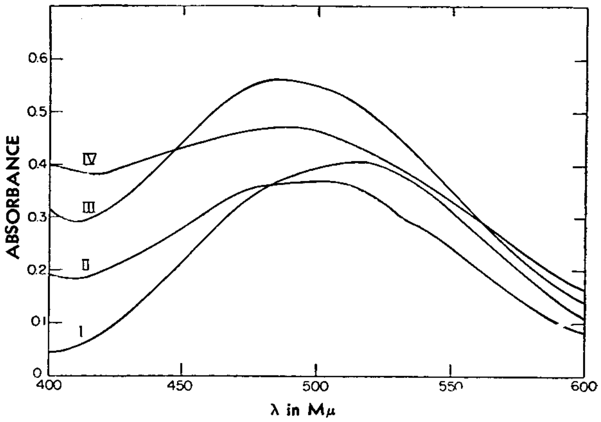

Figure 1. Spectra of phosphate complexes

I. 23.5 mg. manganese per 100 ml. of solution. Obtained by Tomula and Aho also with metaphosphoric acid, pyrophosphoric acid, dihydrogen pyrophosphate, and Calgon
II. 23.5 mg. manganese per 100 ml. of solution, tripolyphosphate as complexing agent
III. 33.5 mg. manganese per 100 ml. of solution, tetrapotassium pyrophosphate as complexing agent
IV. 33.5 mg. manganese per 100 ml. of solution, orthophosphoric acid as complexing agent

Table I. Effect of Final Concentration of Sulfuric Acid and Time on Color Produced in Ortho- and Pyrophosphate Solutions

Final H_2SO_4 Concn., *M*	Color Obtained			
	Pyrophosphate		Orthophosphate	
	Initial	Standing overnight	Initial	Standing overnight
0.1	Colorless	Pale pink	Colorless	Colorless
0.16	Colorless	Magenta	Colorless	Brown ppt.
0.26	Colorless	Magenta	Colorless	Brown ppt.
0.33	Magenta (10 min.)	Magenta	Yellow amber (10 min.)	Brown ppt.
1	Magenta	Magenta	Amber	Brown ppt.
1.5			Amber-red	Brown ppt.
2	Magenta	Magenta	Amber-red	Brown ppt.
3	Magenta	Magenta	Red (some violet)	Brown ppt.
4	Magenta	Magenta		
4.5	Magenta	Magenta	Blood red	Blood red
6	Dark red	Dark red	Dark red	Dark red
9	Dark red	Dark red	Dark red	Dark red
12	Red to yellow (2 min.)	Yellow	Red to yellow (2 min.)	Yellow

pyrophosphate. The results were the same—i.e., acidity was an important positive factor and there was a strong positive interaction between acidity and manganese concentration. Intermediate curves between the absorption curves of the pure phosphate forms were obtained when mixtures of those phosphates were used.

Attempts to determine the combining ratio of manganese with ortho- and pyrophosphate by the method of continuous variations or a molar ratio plot proved futile, because of the formation of manganese dioxide in the absence of excess phosphate.

The results of test tube experiments to determine the effect of the final sulfuric acid concentration on the color produced are given in Table I. The color of the solution on dilution depends on the final acidity. Dilution with sulfuric acid of the same concentration as in the solution resulted in no change of color. The amber-red color of the orthophosphate gave the same spectrum as that for orthophosphate (Figure 1). The spectra of the dark red colors obtained in 6*M* and 9*M* sulfuric acid with both the ortho- and pyrophosphate solutions were the same. This was also the case with the yellow colors in 12*M* sulfuric acid.

The ultraviolet region of the spectrum was examined briefly. Solutions of the manganic orthophosphate complex showed no absorption, but those made from a dihydrogen pyrophosphate in 1*M* sulfuric acid exhibited a peak at 255 mμ, which was fifty-fold higher than the visible peak.

A plot of the sulfuric acid concentration *vs.* absorbance at two manganese concentrations for all of the various phosphates is shown in Figure 2. The comparatively flat region from 7*M* to 9*M* sulfuric acid and the overlapping of all curves in this region suggested that only one complex was present at this concentration of sulfuric acid, regardless of the starting phosphate. The work of Bell (*2*) on the composition of strong phosphoric acids, suggested that all phosphates would be converted to orthophosphate in 8*M* sulfuric acid. In order to test this hypothesis, samples of meta-, pyro-, and tripolyphosphate, containing equal amounts of phosphorus, were dissolved in 8*M* sulfuric acid. After neutralizing with ammonia, the orthophosphate present was determined by the colorimetric method of Fiske and Subbarow (*3*), and calibrated against orthophosphate in 8*M* sulfuric acid. All phosphates showed evidence of complete conversion to orthophosphate. The meta-, pyro-, and tripolyphosphate samples gave no test for orthophosphate unless previously made 8*M* in sulfuric acid. As further confirmation, two solutions of metaphosphate were prepared in which one solution was made 8*M* in perchloric acid, and the other not. Upon neutralizing the acid solution with ammonia, both solutions were analyzed for metaphosphate according to the Jones method (*4*) for precipitating barium metaphosphate at pH 3. The solution which had been acidified showed that metaphosphate was absent.

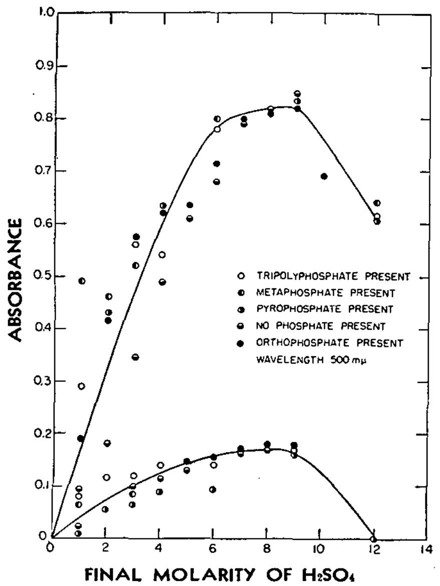

Figure 2. Effect of final sulfuric acid concentration on absorbance of complex

Lower curve, 7 mg. manganese per 100 ml. of solution; upper curve, 35 mg. per ml. of solution. Phosphate concentration, 2.4 millimoles. Solutions above 9*M* in sulfuric acid were measured against reagent blank; in all other cases, the blank was distilled water. Absorbance measurements were made at 500 mμ

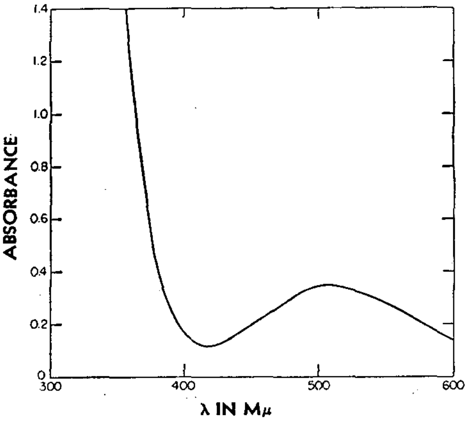

Figure 3. Spectrum of manganic sulfate complex

Manganese concentration, 13 mg. per 100 ml. Sulfuric acid concentration, 8*M*. Blank, distilled water

Because it now appeared that an orthophosphate complex exists in 8*M* sulfuric acid, calibration curves were made and proved to be reproducible. The complex obeyed Beer's law over the range of 2 to 50 mg. of manganese per 100 ml. of solution. Color formation was rapid and suggested the possibility of a molar ratio plot to determine the combining ratio of manganese to orthophosphate. When this was tried, however, the calibration curve was obtained indicating that the complex did not depend on the presence of orthophosphate. A plot of the sulfuric acid concentration *vs.* the absorbance of the complex at two manganese concentrations and containing no phosphate also is included in Figure 2. No red color could be obtained in 8*M* perchloric acid. Absorption spectra and calibration curves run on solutions 8*M* in sulfuric acid and containing no phosphate gave the same curves as obtained under similar conditions with phosphate present. It was therefore concluded that at the high acidities of the method, the sulfate complex predominates.

REAGENTS AND APPARATUS

Sulfuric acid, 18*M* and 8*M*.
Potassium cyanide, 0.5*M*.
Potassium bromate, 0.17*M*.
Manganese stock solution (approximately 3.5 mg. of Mn per ml.); 10 grams per liter of manganous sulfate monohydrate were dissolved in distilled water, and standardized gravimetrically as manganous pyrophosphate.

All measurements of absorbance were made on a Beckman Model DU spectrophotometer using 1-cm. cells.

PROCEDURE

The sample for the determination should contain between 2 and 70 mg. of manganese and should be dissolved (preferably in dilute sulfuric acid) in a volume not over 50 ml. To this sample in a 100-ml. volumetric flask is added an amount of concentrated sulfuric acid calculated to make the solution, upon addition of all reagents, 8*M* in sulfuric acid. Two milliliters of 0.5*M* potassium cyanide and 5 ml. of 0.17*M* potassium bromate are then added and the contents shaken. The flask should be kept stoppered to avoid loss of hydrogen cyanide. The solution is then diluted to the mark with $8M$ sulfuric acid and the absorbance of the solution measured at 500 mμ in a spectrophotometer.

DISCUSSION

The spectrum of the manganic sulfate complex is given in Figure 3. Beer's law is obeyed over the range of 2 to 70 mg. of manganese per 100 ml. of solution, and the absorbance index is about two times that obtained in the Tomula and Aho pyrophosphate method. The standard deviation of the method was estimated to be 0.006 absorbance units from 12 replicate 15-mg. samples run over a period of a month.

In the method bromine is produced both by the oxidation of manganese and by the decomposition of bromate in strongly acid medium. The bromine causes an interfering color which is discharged by the addition of cyanide to form cyanogen bromide.

The colored complex was stable for a week without loss of intensity. Three solutions, containing the same amount of manganic sulfate complex, were prepared using lead dioxide as the oxidizing agent. After excess oxidizing agent was removed, cyanogen bromide was added to one of the solutions, bromate and cyanide to the second solution, and nothing to the third. The solution containing bromate and cyanide was stable for a week, whereas the other two solutions rapidly lost intensity. These findings agree with those of Belcher and West (*1*) and indicate that the stability of the complex is the result of excess bromate in the solution rather than the increased acidity or the presence of cyanogen bromide. The manganic pyrophosphate complex was stable for 12 hours.

The order of reagent addition had no effect on the absorbance of the complex with one notable exception. The manganese must be added to the solution before the bromate, for the cyanogen bromide is not a strong enough oxidizing agent to oxidize manganese to the plus three state.

The temperature at which the color was measured did not have any effect on the absorbance obtained. Most measurements were made with the solutions at room temperature, but even a 20° rise did not affect the reading.

INTERFERENCES

Of the interfering ions bromide, chromic, and cobalt in the original pyrophosphate procedure, only chromic ion is a source of difficulty in the sulfate method. When present at concentrations equal to that of manganese (15 mg. per 100 ml. of solution) arsenous, ferrous, chromic, and stannous ions show a 4% interference. Ions forming insoluble sulfates cause difficulty, but this can be prevented by filtration through a sintered-glass filter funnel. Ions which have been investigated and show no interference at the same concentration as the manganese are cobalt, nickel, zinc, ferric, aluminum, copper, stannic, arsenic, cadmium, bismuth, cerous, ceric, nitrate, fluoride, bromide, and dichromate. For applying the determination in the measurement of recovered carrier, this extent of freedom from interference is more than adequate.

ACKNOWLEDGMENT

The authors are indebted to the Atomic Energy Commission for partial financial support.

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RECEIVED for review May 14, 1954. Accepted November 2, 1954.

Molybdenum Blue Reaction and Determination of Phosphorus in Waters Containing Arsenic, Silicon, and Germanium

HARRY LEVINE, J. J. ROWE, and F. S. GRIMALDI
U. S. Geological Survey, Washington 25, D. C.

Microgram amounts of phosphate are usually determined by the molybdenum blue reaction, but this reaction is not specific for phosphorus. The research established the range of conditions under which phosphate, arsenate, silicate, and germanate give the molybdenum blue reaction for differentiating these elements, and developed a method for the determination of phosphate in waters containing up to 10 p.p.m. of the oxides of germanium, arsenic(V), and silicon. With stannous chloride or 1-amino-2-naphthol-4-sulfonic acid as the reducing agent no conditions were found for distinguishing silicate from germanate and phosphate from arsenate. In the recommended procedure the phosphate is concentrated by coprecipitation on aluminum hydroxide, and coprecipitated arsenic, germanium, and silicon are volatilized by a mixture of hydrofluoric, hydrochloric, and hydrobromic acids prior to the determination of phosphate. The authors are able to report that the total phosphorus content of several samples of sea water from the Gulf of Mexico ranged from 0.018 to 0.059 mg. of phosphorus pentoxide per liter of water.

THE literature on the determination of phosphorus by the molybdenum blue reaction is voluminous. The methods are based on the formation of molybdophosphoric acid and its subsequent reduction to a blue compound. The original Denigès method (*1*) has been modified for the determination of phosphorus in sea water. Important papers on water analysis include those of Zinzadze (*8*), Kalle (*4*), Redfield *et al.* (*5*), Woods *et al.* (*7*), Harvey (*3*), and Robinson *et al.* (*6*).

The molybdenum blue reaction is not specific for phosphorus because arsenic (V), germanium, and silicon also form heteropoly acids with molybdenum, which also yield blue compounds on reduction. Some selectivity for phosphorus may be obtained by control of acidity. For example, at high acidity the heteropoly acids of phosphorus and arsenic may be reduced without interference from small amounts of silicon and germanium. The interference of arsenate in sea water may be eliminated by reducing the arsenate to arsenite before the addition of ammonium molybdate. Various agents—such as sodium hydrogen sulfite—have been proposed for the reduction of arsenic, but the literature contains conflicting statements as to their effectiveness. It is also reported that arsenite may enhance the intensity of the blue color of the reduced molybdophosphoric acid.

Various reducing agents have been employed for the reduction of molybdophosphoric acid, so as to increase the stability of the resulting molybdenum blue complex and to make the reaction less sensitive to disturbing ions.

The first aim of this research sought to establish the conditions under which phosphorus, arsenic, silicon, and germanium give the molybdenum blue reaction. The data should increase the knowledge of the chemistry of the heteropoly acids of these elements and should reveal differences in behavior that may be of analytical significance. The second aim was to develop a method for the determination of microgram amounts of phosphorus applicable to waters containing arsenate, silicate, and germanate.

STUDIES OF MOLYBDENUM BLUE REACTION

The variables studied were the acidity, molybdate concentration, and concentration of the reducing agent. Two series of experiments were made. Stannous chloride was used as the reducing agent in one series and 1-amino-2-naphthol-4-sulfonic acid, introduced by Fiske and Subbarrow (*2*), in the other. These reducing agents were chosen on the basis of their present popularity and are representative of an inorganic and an organic reducing agent.

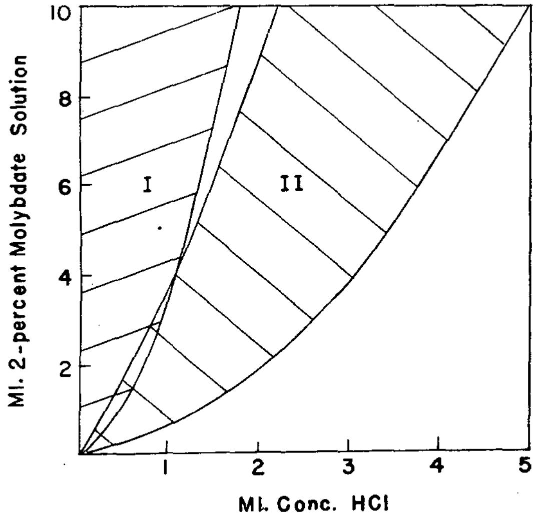

Figure 1. Molybdenum blue fields with 1-amino-2-naphthol-4-sulfonic acid as reducing agent

I. Silicon or germanium
II. Phosphorus or arsenic

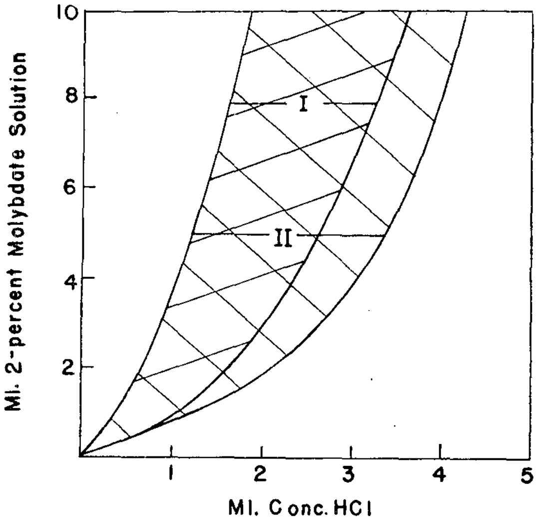

Figure 2. Molybdenum blue fields with stannous chloride as reducing agent

I. Silicon or germanium
II. Phosphorus or arsenic

Procedure. The following general procedure was used in this study. Five milliliters of one solution (10 γ of phosphorus pentoxide, 25 γ of arsenic pentoxide, 25 γ of silicon dioxide, or 25 γ of germanium dioxide) were added to a 100-ml. beaker. Next a known amount of hydrochloric acid was added, and the solution diluted to 25 ml. with distilled water. The molybdate solution in known amounts was then added, and the volume adjusted to 45 ml. with water. Finally, a known amount of reducing agent was added and the volume adjusted to 50 ml. In the experiment where stannous chloride was used as the reducing agent, the absorbance of the resulting solution was measured at 735 mμ after 3 minutes at room temperature using 1-cm. cells, slit at 0.06 mm. (effective band width 3.3 mμ), and water as reference. The same procedure was used for 1-amino-2-naphthol-4-sulfonic acid when silicon and germanium test solutions were used. Test solutions containing phosphorus and arsenic, however, were heated just to boiling and cooled before measuring the absorbance. This heating was necessary in the cases of phosphorus and arsenic because the reduction of the heteropoly acids proceeded too slowly at room temperature with the organic reductant.

Molybdate concentration and acidity were studied simultaneously. Forty combinations were used, in which the amounts of molybdate solution were varied from 0.5 to 10 ml. in increments of about 2 ml., and concentrated hydrochloric acid varied from 0.02 to 5 ml. in increments of about 1 ml.

Following a similar representation used by Harvey (*3*), the fields representing the conditions under which the molybdenum blue reactions take place are given in Figures 1 and 2. The amount of hydrochloric acid plotted on the abscissa includes the hydrochloric acid derived from the stannous chloride reagent when this reductant is used. The data for silicon and germanium are plotted together because the same data are obtained for both elements. This is also true for arsenic and phosphorus. The molybdenum blue reaction is assumed to occur only if the blank shows a very small absorbance (<0.01) and if the sample shows a visible blue and a greater absorbance than the blank. The reactions obtained were dependent on the amount of reducing agent used. The figures show a composite of the data obtained for several concentration levels of each reducing agent. The amount of the 1-amino-2-naphthol-4-sulfonic acid reductant ranged from 0.1 to 2 ml., and tests were made with 0.1, 0.5, 1, and 2 ml. of the reductant for each combination of molybdate and hydrochloric acid concentration. The stannous chloride was varied from 0.1 to 3 ml., and tests were made with 0.1, 0.3, 1, and 3 ml. of stannous chloride solution for each combination of molybdate and hydrochloric acid concentration.

The concentration of the organic reductant is relatively unimportant in the germanium reaction; the same molybdenum blue field was obtained for the various concentrations of reductant tested. The concentration of the reductant is slightly more important for arsenate; 0.1 ml. of reductant is more nearly optimum. The fields of reactivity for silicate and phosphate are spotty and the concentration of reductant is more critical for both. The optimum amount of organic reductant for silicate should be between 1 and 2 ml., and for phosphate between 0.5 and 1 ml.

The molybdenum blue reactions for silicate and germanate were found to be especially sensitive to changes in concentration of the stannous chloride, and for this reason stannous chloride is not an ideal reagent for these two elements. The molybdenum blue field was exceptionally spotty, and a reaction occurred under only a few of the conditions. If this reagent is used, about 0.1 ml. of stannous chloride solution should be nearly optimum for either element. Arsenate is least sensitive to stannous chloride concentration, and the optimum amount of the reductant should be between 0.1 and 1 ml. of reagent. Stannous chloride seems to be better than the 1-amino-2-naphthol-4-sulfonic acid for the reduction of molybdophosphoric acid. The field of reactivity is again somewhat spotty unless the amount of stannous chloride is within the range of 0.1 to 0.5 ml.

The conditions for which the molybdenum blue reactions take place depend on the particular reducing agent chosen; for the reducing agents tested, silicate cannot be differentiated from germanate, nor can phosphate be differentiated from arsenate. It is unlikely that such differentiations can be made with some other reducing agent. However, one reductant may be better than another for purposes of differentiating the pair arsenic and phosphorus from the pair germanium and silicon. In this respect the 1-amino-2-naphthol-4-sulfonic acid is better than the stannous chloride. The advantage is not as clear cut for the 1-amino-2-naphthol-4-sulfonic acid as is indicated in Figure 2, for other reactions producing foreign colors upset this balance. In the following work, stannous chloride was chosen as the reducing agent.

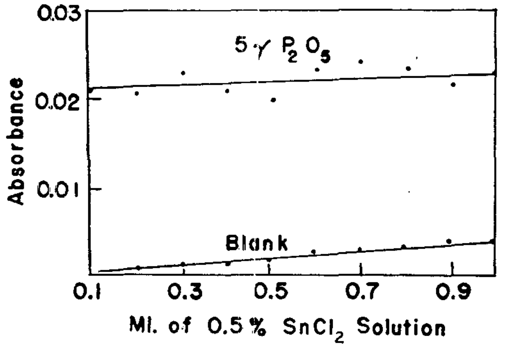

Figure 3. Effect of stannous chloride concentration

Solutions, 50 ml., containing 5 ml. of 2% molybdate solution and 2.5 ml. of concd. HCl

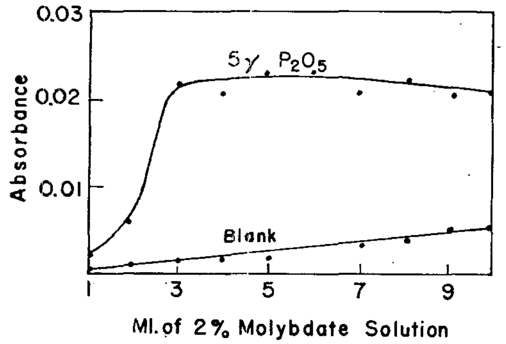

Figure 4. Effect of molybdate concentration

Solutions, 50 ml., containing 0.3 ml. of 0.5% stannous chloride solution and 2.5 ml. concd. HCl

OPTIMUM CONDITIONS

The molybdenum blue color should be most stable and the reaction least sensitive to small changes in conditions for concentrations of reagents represented by some point near the center of the molybdenum blue field (Figure 2). Accordingly, this region was investigated in greater detail to establish optimum working conditions. The described general procedure was used to obtain the data. In each experiment 5 γ of phosphorus pentoxide were taken, and the final volume of each solution was made to 50 ml. Figure 3 illustrates the effects produced by varying the stannous chloride concentration in the solution containing 2.5 ml. of hydrochloric acid and 5 ml. of molybdate reagent. The optimum amount of stannous chloride solution was taken as 0.3 ml. The data plotted in Figure 4 illustrate the effect of varying the molybdate concentration, when the total amount of hydrochloric acid is fixed at 2.5 ml. and stannous chloride solution is fixed at 0.3 ml. Five milliliters of molybdate reagent were taken as optimum. In Figure 5, the acidity was varied in solutions at optimum concentrations of stannous chloride and ammonium molybdate. The optimum amount of hydrochloric acid is 2.5 ml. or a concentration of 5% by volume. The spectral transmittance curve, Figure 6 shows an optimum wave length at 735 mμ. Under the optimum conditions determined, a straight-line relationship is obtained between absorbance and phosphate concentration up to 80 γ of phosphorus pentoxide per 50 ml., the maximum concentration tested (an absorbance of 0.35 is given by this solution). The absorbances of the standards were redetermined after allowing the solutions to stand for 1 hour; the absorbances decreased by an average of 5%.

BEHAVIOR OF ARSENIC, GERMANIUM, AND SILICON

The interference of arsenic (III), arsenic (V), germanium, and silicon was checked, under optimum conditions for phosphate, by determining the absorbance of 25 γ of each of the oxides of these elements alone and in the presence of 5 γ of phosphorus pentoxide. The absorbances were additive. The extent of interference of these elements is summarized as follows:

Oxides, 25 γ	P_2O_5 Equiv., γ
As_2O_5	8
As_2O_3	<1
GeO_2	2.5
SiO_2	2

DETERMINATION OF PHOSPHORUS IN WATERS CONTAINING ARSENIC, GERMANIUM, AND SILICON

The procedure provides for the separation of phosphorus from at least 1 mg. each of arsenic pentoxide, germanium dioxide, and silicon dioxide per 100 ml. of solution prior to the determination of phosphorus by the molybdenum blue reaction using stannous chloride as the reducing agent. The phosphate in sea water is concentrated by precipitating aluminum hydroxide, which serves as a carrier for the aluminum phosphate formed. The small precipitate is collected by centrifuging. Filtration cannot be used because filter paper ash contains appreciable amounts of phosphate. The precipitate is treated with a small volume of solution containing hydrofluoric, hydrobromic, hydrochloric, and sulfuric acids to volatilize any coprecipitated arsenic, germanium, and silicon. The phosphate is then determined by the molybdenum blue reaction.

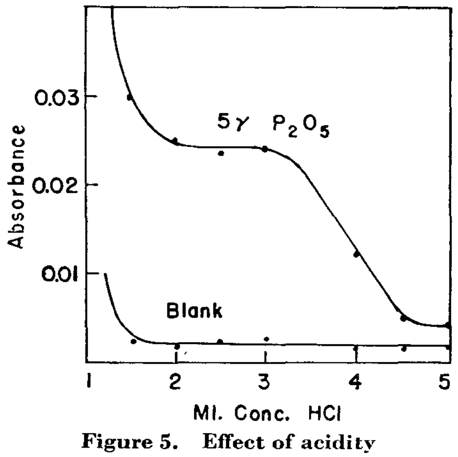

Figure 5. Effect of acidity

Solutions, 50 ml., containing 0.3 ml. of 2% stannous chloride solution and 5 ml. of 2% molybdate solution

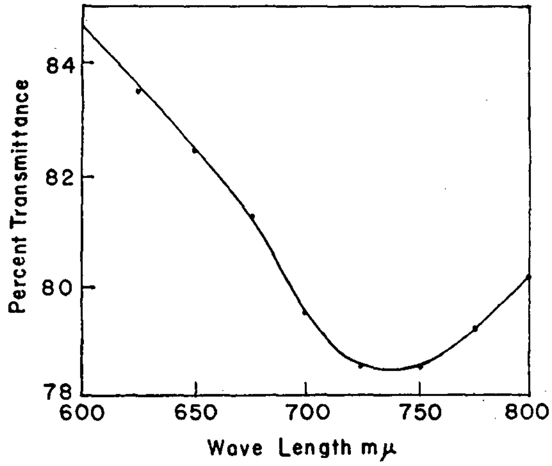

Figure 6. Spectral transmittance of molybdenum blue solution

Solution, 50 ml., containing 20 γ P_2O_5, 2.5 ml. of HCl, 5 ml. of 2% molybdate solution, 0.3 ml. of 0.5% $SnCl_2$. Water as reference. Slit 0.06 mm.

Table I. Recoveries of Phosphate after Volatilization of $AsCl_3$, $GeCl_4$, and SiF_4 (Series 1)

Taken				P_2O_5, Found, γ
P_2O_5, γ	As_2O_5, mg.	GeO_2, mg.	SiO_2, mg.	
10.0	0.01	..	..	10.2
30.0	0.01	..	..	30.4
10.0	0.10	..	..	10.0
30.0	0.10	..	..	30.0
10.0	1.0	..	..	10.7
30.0	1.0	..	..	30.9
10.0	..	..	0.01	10.2
30.0	..	..	0.01	31.0
10.0	..	..	0.10	9.8
30.0	..	..	0.10	30.5
10.0	..	..	1.0	11.0
30.0	..	..	1.0	31.5
10.0	..	0.01	..	10.5
30.0	..	0.01	..	31.6
10.0	..	0.10	..	10.6
30.0	..	0.10	..	30.5
10.0	..	1.0	..	10.7
30.0	..	1.0	..	32.0

PROCEDURE

All stock solutions were stored in polyethylene bottles.

Reducing agent solutions were prepared fresh each day.

Phosphate, Arsenate, and Germanate Solutions. Dissolve sodium or potassium salts in water redistilled in glass stills.

Reagents and Apparatus. Standard Phosphate Stock Solution (1 ml. contains 0.0001 gram of phosphoric anhydride). Dissolve 0.1917 gram of potassium dihydrogen phosphate in redistilled water to give 1 liter of solution.

Standard Phosphate Solution (1 ml. contains 2 γ of phosphoric anhydride). Take 10 ml. of phosphate stock solution and dilute with redistilled water to 1 liter.

Standard Silicate Solution. Dissolve anhydrous silicon dioxide in sodium hydroxide.

Stannous Chloride Stock Solution [5% in hydrochloric acid (1 to 19)]. Dissolve 5 grams of stannous chloride dihydrate in 10 ml. of hot hydrochloric acid (1 to 1), and dilute to 100 ml. with water. This solution may be kept for about a week.

Stannous Chloride Solution (0.5%). Take 10 ml. of stannous chloride stock solution and dilute to 100 ml. with water. A fresh solution is prepared daily.

Ammonium Molybdate Solution (2%). Dissolve 2 grams of ammonium molybdate, $(NH_4)_6Mo_7O_{24}.4H_2O$, in water to give 100 ml. of solution.

Aluminum Chloride Solution (1 ml. contains 0.002 gram of aluminum oxide). Dissolve 0.95 gram of aluminum chloride hexahydrate in water to give 100 ml. of solution.

Ammonium Chloride Wash Solution (0.1%). Dissolve 0.5 gram of reagent grade ammonium chloride in water to make 500 ml. of solution.

Ammonium Hydroxide Solution. Bubble tank ammonia into doubly distilled water.

Hydrochloric Acid (36%), Hydrobromic Acid (48%), Sulfuric Acid (96%), and Hydrofluoric Acid (46%). All were reagent grade.

Table II. Recoveries of Phosphate from Distilled and Ocean Waters

	1 P_2O_5 in Sample, γ	2 P_2O_5 Added, γ	3 As_2O_5 Taken, Mg.	4 GeO_2 Taken, Mg.	5 SiO_2 Taken, Mg.	Total P_2O_5, γ	
						Col. 1 + 2	Found
Series 2	..	5.0	0.01	0.01	0.01	5.0	5.0
	..	20.0	0.01	0.01	0.01	20.0	18.8
	..	5.0	0.10	0.10	0.10	5.0	5.3
	..	20.0	0.10	0.10	0.10	20.0	19.0
	..	5.0	1.0	1.0	1.0	5.0	5.2
	..	20.0	1.0	1.0	1.0	20.0	20.0
Series 3	1.7	3.3	0.01	0.01	0.01	5.0	5.3
	1.7	18.3	0.01	0.01	0.01	20.0	18.5
	1.7	3.3	0.02	0.02	0.02	5.0	4.5
	1.7	18.3	0.02	0.02	0.02	20.0	19.2
	1.7	3.3	0.03	0.03	0.03	5.0	5.0
	1.7	18.3	0.03	0.03	0.03	20.0	20.1
	1.7	3.3	0.05	0.05	0.05	5.0	5.3
	1.7	18.3	0.05	0.05	0.05	20.0	19.8
	1.7	3.3	0.10	0.10	0.10	5.0	5.5
	1.7	18.3	0.10	0.10	0.10	20.0	19.3
	1.7	3.3	1.0	1.0	1.0	5.0	6.8
	1.7	18.3	1.0	1.0	1.0	20.0	19.5

1-Amino-2-Naphthol-4-Sulfonic Acid Solution. Add 1.5 grams of the reagent to 200 ml. of water containing 7 grams of anhydrous sodium sulfate. Mix the resulting solution with this following solution: dissolve 40 grams of sodium pyrosulfite ($Na_2S_2O_5$) in 800 ml. of water.

Water doubly distilled from a borosilicate glass still was used for all dilutions and for the preparation of reagents.

The spectrophotometer used was the Beckman Model DU, with 1-cm. Corex cells.

The International centrifuge Model V, size 2, was used.

Determination of Phosphorus. Pipet 100 ml. of sea water into a clean porcelain dish, and evaporate the solution to dryness on the steam bath.

Destroy organic matter by placing the dish in a furnace and heating for about 30 minutes at 600° C. Cool.

Add 6 ml. of hydrochloric acid (1 to 1) and digest the solution. Add 35 ml. of water, and digest again to dissolve salts.

Add 10 ml. of aluminum chloride solution, heat to a gentle boil, and add ammonium hydroxide until methyl red is just yellow. Digest the solution on the bath for a few minutes. Cool.

Transfer the contents to a centrifuge tube, and centrifuge for 10 minutes at 1500 to 2000 r.p.m. Decant clear solution. Wash the precipitate twice by centrifuging with two 15-ml. portions of 0.1% ammonium chloride solution, discarding the clear solutions.

Transfer the precipitate to a small platinum dish. Add several drops of hydrochloric acid and 1 ml. of hydrofluoric acid, and evaporate to dryness on the steam bath.

Add 5 ml. of hydrochloric acid and evaporate to dryness.

Add 1 to 2 ml. of hydrochloric acid and 5 ml. of water, digest to dissolve salts, and transfer the solution to a small Erlenmeyer flask. Add 1 ml. of hydrobromic acid and 0.2 ml. of sulfuric acid and evaporate to fumes of sulfur trioxide.

Fume gently for a few minutes to remove any hydrobromic acid and bromine. Cool.

Add 2.00 ml. of hydrochloric acid and wash sides of flask down

Table III. Phosphate Content of Sea Water from Gulf of Mexico

(Samples taken June 12, 1952)

Sample No.	Location	Sample Depth, Meters	P_2O_5, Mg./Liter
55	Station 15 (water depth 100 fathoms) Lat. 26°08′ Long. 84°19′	50	0.020 0.022
57	Station 15 (water depth 100 fathoms) Lat. 26°08′ Long. 84°19′	140	0.022 0.024
58	Station 15 (water depth 100 fathoms) Lat. 26°08′ Long. 84°19′	182	0.055 0.063
59	Station 16 (water depth 100 fathoms) Lat. 26°47′ Long. 84°33′	0	0.017 0.019
60	Station 16 (water depth 100 fathoms) Lat. 26°47′ Long. 84°33′	25	0.018 0.019

with water. Add water to make to 30-ml. volume and digest the solution a few minutes to obtain a clear solution. Cool.

Transfer the solution to a 50-ml. volumetric flask. Add 5 ml. of ammonium molybdate solution. Adjust volume to 45 ml. and mix.

Add 0.3 ml. of stannous chloride. Make to volume and mix.

Measure the absorbance of the solution within 30 minutes with a Beckman DU spectrophotometer at 735 mμ. Use 0.06-mm. slit, setting water at 100% transmittance.

Obtain phosphorus content by reference to a standard curve. At least one blank should be run at the same time with the samples and its value deducted.

Test of Procedure. Three series of experiments were made to test the procedure. In the first series mixtures of phosphate and arsenate, phosphate and germanate, and phosphate and silicate in 60 ml. of distilled water were tested. In the second series distilled water, to which were added various amounts of phosphate, arsenate, silicate, and germanate, was used. In the third series a sample of sea water from the Gulf of Mexico was used to which were added various amounts of the same elements. The total phosphorus content of the sea water was first established by quintuplet determinations. The values found in 100 ml. of sea water were 1.9, 1.5, 1.6, 1.9, and 1.6 γ (average 1.7 γ) after subtracting an average blank of 4 γ. The results given in Tables I and II indicate good recoveries of added phosphate.

PHOSPHATE CONTENT OF SEA WATER FROM GULF OF MEXICO

Total phosphorus was determined in duplicate on five samples of sea water collected from the Gulf of Mexico. The values found, given in Table III, ranged from 0.018 to 0.059 mg. of phosphoric anhydride per liter of water.

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Received for review June 29, 1954. Accepted October 9, 1954. Publication authorized by the Director, U. S. Geological Survey. Work done on behalf of the Division of Raw Materials, U. S. Atomic Energy Commission.

Method for Evaluating the Reliability of Routine Molecular Weights Determined by Light Scattering

S. V. R. MASTRANGELO[1], BARBARA CLAY, M. M. FISHMAN[2], A. G. HAGAN,
ALLAN LAZRUS, and WALTER ZAGAR

The Dextran Corp., Yonkers 1, N. Y.

A method has been devised for evaluating the reliability of routine molecular weights determined by light scattering. The material studied was clinical dextran. The method consists of correlating: (*a*) the molecular weight of the low 10% molecular weight fraction against the volume per volume per cent of methanol necessary to precipitate 91.0% of the clinical dextran fraction and (*b*) log [η] against the weight average molecular weight of the whole clinical fraction. These data are used to check light-scattering results. The weight average molecular weight of the low 10% fraction is then used with the weight average molecular weight of the whole clinical fraction to calculate the weight average molecular weight of the high 10% fraction by means of a modified Lansing-Kraemer distribution function. This is then used as a check on the observed molecular weight of the high 10% fraction determined by light scattering.

THE great interest shown by the armed forces in the use of dextran as a blood plasma expander has led to the formulation of a set of government specifications covering its purchase. These specifications are based on work done at the Northern Regional Research Laboratory, in Peoria, Ill., the National Bureau of Standards, Washington, D. C., and by various interested industrial groups (*7*). One of the specifications calls for the measurement of the weight average molecular weight of the whole clinical fraction as well as of the high and low 10% fractions. The molecular weights are measured by means of light scattering. During normal plant operation, this may require as many as 10 to 16 molecular weights to be determined daily. A routine such as this, when carried out under the pressure exerted by the plant due to the accumulation of back samples, makes it rather difficult to check the results by duplicate or triplicate sets of measurements. Yet, it is exceedingly important that each result be reliable, inasmuch as the final product is for clinical use.

In order to evaluate the reliability of the results and, at the same time, to continue at a high level of production, it became necessary to invent a scheme for cross-checking molecular weights belonging to a particular batch by making use of fractionation and viscosity data. The authors believe this method to be of general utility for the routine determination of the molecular weight distributions of a polymer product by measurement of the weight average molecular weight of the low, average, and high molecular weight fractions. This type of information is, of course, very useful for purposes of characterizing and specifying polymer products.

The scheme proposed for providing a reference frame to check molecular weight data is based on three observations:

1. The weight average molecular weight of a low 10% fraction of a polymer can usually be related, mathematically, to the volume per cent of precipitant required to remove all but this fraction from solution. Once sufficient data are obtained to establish the relationship between the weight average molecular weight and the volume per cent of precipitant (at constant temperature and original concentration of polymer) required to precipitate all but the low 10% fraction from solution, the fractionation data obtained on future batches will serve to give a value of the weight average molecular weight which can then be compared with the value obtained by light scattering. The fractionation data give, in effect, the equivalent of a duplicate determination.
2. The weight average molecular weight of the whole polymer over a small range may usually be correlated against inherent viscosity.
3. The same mathematical law describes the distribution of molecular weight in each batch, save for one or two constants which may vary from batch to batch. The nature of the polymer will usually permit the proper selection of distribution function to be made (*1*). The molecular weight of the low 10% fraction and the molecular weight of the average fraction, as verified by

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[1] Present address, Barrett Division, Allied Chemical and Dye Corp., Philadelphia, Pa.

[2] Present address, Department of Chemistry, The City College of New York, New York, N. Y.

observations 1 and 2, may then be used to calculate the one or two constants of the distribution function. From this function, the weight average molecular weight of the high 10% fraction may then be calculated. This value may now be compared with the experimental value.

APPARATUS AND PROCEDURE

The apparatus used for the light-scattering measurements consisted of a Brice and Speiser light-scattering photometer (*3*) and a Brice and Speiser differential refractometer (*4*). The light-scattering procedure used is based on work done at the Northern Regional Research Laboratory, the National Bureau of Standards, and other interested laboratories. Selas 04 filter candles encased in glass envelopes were used for preparing optically clear solutions for light scattering by pressure filtration.

The samples were prepared by filtering under 15-pound pressure through a Selas 04 filter candle directly into a light-scattering dissymmetry cell. The candle is first flushed with distilled water until the measured dissymmetry, I_{45}/I_{135}, is less than 1.5 using 436-mμ light, and the measured turbidity is less than 6.0×10^{-5} cm.$^{-1}$ The turbidity of the water is then recorded for use in making the background correction. The filter is rinsed with a 0.2% solution of the dextran and flushed into the same dissymmetry cell used previously to measure the turbidity of the clarified distilled water. The cell is rinsed with this preliminary filtrate and filled with about 50 ml. of fresh filtrate. If the measured dissymmetry is less than 1.08, the solution is ready for scattering; otherwise, the solution is refiltered until a dissymmetry of 1.08 or less is obtained. Several filtrations may be required to accomplish this. It is necessary to work with solutions of low dissymmetry, since, as was shown by Senti and Hellman (*10*), the dissymmetry of the average clinical dextran molecule is close to 1.00 and therefore, any higher value indicates insufficient optical clarity. The values of the turbidity and dissymmetry are recorded, and the procedure is repeated for three more dilutions, making in all, four solutions at concentrations of 0.2, 0.4, 0.6, and 0.8%, respectively.

The turbidity, τ, at each of the four concentrations, c, is calculated; corrections for the background scattering are made by subtracting the turbidity of the water from the turbidity at each of the four concentrations; and then the values of $(H\frac{c}{\tau})$, where H is a constant whose value was derived by Debye (*5*), are least squared with c to the linear Equation 1.

$$(H\tfrac{c}{\tau}) = \frac{1}{M} - (2B)c \qquad (1)$$

where M is the weight average molecular weight and B is a virial constant. The reciprocal of the intercept is computed and recorded as the weight average molecular weight, providing that not more than one point in four deviates from linearity by more than 5%.

AVERAGE MOLECULAR WEIGHT

Over a small range (60,000 to 100,000) of the entire clinical fraction, a relationship exists between the weight average molecular weight obtained by light scattering and the inherent viscosity, $[\eta]$. This type of relationship has been described by Hofreiter (*6*) for enzymatic dextran.

Table I. Correlation of Weight Average Molecular Weight of Whole Clinical Fraction with Inherent Viscosity

	$[\eta]$	Av. M_w by L.S.	Av. M_w by $[\eta]$	Deviation Grams/mole	Deviation %
1	0.227	56,800	55,400	+1,400	+2.5
2	0.235	66,000	60,600	+5,400	+8.9
3	0.239	61,300	63,300	−2,000	−3.2
4	0.241	68,900	64,700	+4,200	+6.5
5	0.243	62,700	66,300	−3,500	−5.3
6	0.246	65,800	68,200	−2,400	−3.5
7	0.248	71,900	69,700	+2,200	+3.2
8	0.251	75,300	71,800	+3,500	+4.9
9	0.254	73,500	74,000	− 500	−0.7
10	0.257	81,000	76,300	+4,700	+6.2
11	0.260	78,400	78,600	− 200	−0.3
12	0.263	79,900	81,000	−1,100	−1.4
13	0.265	80,200	82,500	−2,300	−2.8
14	0.266	78,800	83,400	−4,600	−5.5
15	0.269	84,500	85,800	−1,300	−1.5
16	0.270	87,000	86,600	+ 400	+0.5
17	0.272	81,300	88,300	−7,000	−7.9
18	0.274	94,900	90,000	+4,900	+5.4
19	0.278	92,400	93,300	− 900	−1.0
20	0.280	89,300	95,100	−5,800	−6.1

The determination of the weight average molecular weight of the whole clinical fraction usually presents little or no difficulty, since, once autoclaved, the material is never subject to the influence of nonsolvents, heat, concentration by evaporation, and other factors causing aggregation or disaggregation in solution. Therefore the weight average molecular weight of the whole clinical fraction is usually as reliable as the accuracy of the instrument used for the measurement.

Since this is so, the mathematical relationship between weight average molecular weight and inherent viscosity for the product used was established by the method of least squares on fifty whole culture batches. This relationship is expressed as Equation 2.

$$\log M_w = 2.5714 \log[\eta] + 6.3998 \qquad (2)$$

The equation was tested on subsequent batches and, in Table I, are listed some of the correlations obtained through the use of this equation.

The average deviation in the table is ±3.86%.

FRACTIONATION PROCEDURE

The fractionation procedure used for preparing the high and low 10% fractions of clinical dextran is based on an unpublished scheme proposed by Weissberg (*14*). The starting material was clinical dextran prepared by careful fractionation of crude material under fixed conditions. A 6.0 ± 0.3% solution of this clinical dextran in 0.9% saline was prepared and autoclaved for 30 minutes at 15-pound pressure. Six portions of 100 ml. of the clinical dextran solution are pipetted into each of six 250-ml. centrifuge bottles and from a 100-ml. automatic buret, increasing amounts of 99.85% synthetic methanol precipitant are added successively to each. Amounts ranging from 74 to 113 ml. of precipitant were found by experience to precipitate from 5 to 95% of the dextran. The bottles are capped, rotated to mix in such a way as not to lose any of the sample, and placed in a water bath at 40° ± 5° C. for 30 minutes to effect homogeneity and then, in a water bath at 25° ± 0.05° C. for 1 hour to effect equilibrium at constant temperature. The bottles are removed from the bath at 25° C. after 30 minutes, rotated, and returned to the bath in order to ensure temperature equilibrium. The bottles are then removed from the bath, and centrifuged for 15 minutes in an International centrifuge at 2000 r.p.m. The centrifuge is kept in a constant temperature room maintained at 24.0° ± 1.0° C. and has its lid perforated at random with 0.25-inch holes to allow for better circulation of air.

The centrifuge bottles are removed from the centrifuge and the supernatant liquid is taken off by suction. The precipitates (gels) are quantitatively transferred to volumetric flasks of appropriate size, depending on the size of the precipitate. The volumetric flasks are made to volume and the amount of material in each precipitate is determined rapidly by means of a polarimeter, using $[\alpha]_D^{20} = 199$ (*11*), or by means of a differential refractometer, using $\frac{dn}{dc} = 0.151$ (*13*). These data are then used to obtain the plot of per cent of material precipitated versus the volume per volume per cent of methanol added. From this precipitation guide curve, the amounts of methanol required to precipitate 10.0 and 90.0% of the dextran are calculated and used for obtaining, respectively, the high molecular weight 10.0% precipitate and supernatant containing the 10.0% fraction on the low molecular weight side. The low molecular weight fraction is then removed from solution by adding in excess of 85 volume per volume % of methanol. The two molecular weight fractions are stored in the dry form prepared by lyophylization.

LOW MOLECULAR WEIGHT FRACTION

According to Boyer (*2*) and Weissberg and coworkers (*13*), it should be possible, in theory, to correlate the amount of material precipitating with various volumes of precipitant added against the weight average molecular weight of the precipitate. However, in practice, the high molecular weight fractions are usually too sensitive to temperature, amount of precipitant added, original concentration of polymer, molecular weight of low molecular weight fractions remaining in solution, and other factors not easily controlled, to be accurately correlated against methanol precipitability. This was found to be particularly true for dextran systems. The method of keeping the volume per volume per cent of methanol constant and varying the temperature has been used, incidentally, by Wales (*12*), but the accuracy is not good. Fortunately, many of the difficulties are removed when it is desired to correlate the low molecular weight fractions against methanol precipitability—e.g., temperature and solvent sensitivity are usually within controllable limits, relatively insensitive to original polymer concentration since most of the polymer is now precipitated, and interfering molecular weight species are no longer present in solution.

The molecular weight (extrapolated to 10% by an empirical method) of 15 consecutive low 10% fractions of clinical dextran was plotted against the volume per volume per cent of methanol required to precipitate 91.0% (a figure arbitrarily selected to assure precipitation of fractions not less than 90%, to meet government specifications) of the clinical dextran from a 6.0 ± 0.3% solution at 25.00° ± 0.05° C. Equation 3 was obtained by fitting the data to a straight line by the method of least squares.

$$M_w = 4{,}907.4\,P + 276{,}725 \qquad (3)$$

where Mw is the weight average molecular weight of the low 10% fraction, and P is the volume per volume per cent of methanol required to precipitate 91.0% of the dextran from a 6.0 ± 0.3% solution at 25.00° ± 0.05° C. Table II lists the values of the weight average molecular weight of the low 10.0% fractions obtained by light scattering and the values calculated by Equation 3 using the precipitation guide curves to obtain the values of P. The agreement is seen to be good to 5% in the worst case and about 2% on the average. This is certainly within the accuracy of the light scattering measurements. Thus, the calculated value serves as a check on the determination of the molecular weight by light scattering.

HIGH MOLECULAR WEIGHT FRACTIONS

The two reliable values of the molecular weight of the low 10.0% fractions and the whole clinical fraction may now be used to calculate the molecular weight of the high 10.0% fraction, or a suitable reliability factor.

For the whole culture dextran, a Lansing-Kraemer (*8*) type of distribution function was used. Since a slight modification of this function was necessary to account for removal of a top, as well as of a bottom fraction from the polymer, a brief description of the treatment is given.

The usual form of the Lansing-Kraemer function is shown by Equations 4 and 5.

$$W(M) = Ke^{-y^2} \qquad (4)$$

$$y = \frac{1}{\beta} \ln \frac{M}{M_0} \qquad (5)$$

where $W(M)$ is the differential weight distribution function, β and M_0 are constants, and $1/K = \beta\ M_0\ (\pi)^{1/2}\ e^{\beta^2/4}$. The usual application of this method requires a knowledge of M_w, M_n, and Mz to evaluate the constants M_0 and β by making use of the relationships shown in Equations 6,7, and 8.

$$M_n = M_0\, e^{0.25\beta^2} \qquad (6)$$

$$M_w = M_0\, e^{0.75\beta^2} \qquad (7)$$

$$M_z = M_0\, e^{1.25\beta^2} \qquad (8)$$

However, because the authors wished to calculate the weight average molecular weight of a high 10% fraction from a knowledge only of the M_w of the whole polymer and the weight average molecular weight, $M_w(P)$, of the low P% fraction of this polymer they derived the form shown by Equation 9.

$$M_w(P) = \frac{M_0 e^{0.75\beta^2} \frac{1}{\sqrt{\pi}} \int_{-\infty}^{z+\beta} e^{-z^2}\, dz}{\frac{1}{\sqrt{\pi}} \int_{-\infty}^{z+\beta/2} \epsilon^{-z^2}\, dz} \qquad (9)$$

Table II. Correlation of Weight Average Molecular Weight of Low 10% Fraction of Clinical Dextran with Methanol Precipitation

	V./V.%' Methanol to Precipitate 91.0% of Clin. Dextran Fraction	M_w of Low 10.0% Fraction of Clinical Dextran		Deviation	
		Observed	Calculated[a]	Grams/mole	%
1	52.50	19,900	19,100	+800	+4.5
2	51.10	25,800	25,900	−100	−0.4
3	51.30	25,800	25,000	+800	+3.1
4	50.95	27,300	26,700	+600	+2.2
5	48.40	40,200	39,300	+900	+2.2
6	49.45	34,200	34,000	+200	+0.6
7	48.55	38,600	38,600	0	0.0
8	48.90	37,500	36,800	+700	+1.9
9	50.45	28,900	29,100	−200	−0.7
10	49.95	30,400	31,600	−1,200	−3.9
11	49.10	34,900	35,700	−800	−2.3
12	49.45	33,900	34,000	−100	−0.3
13	51.35	25,100	24,700	+400	+1.6
14	51.35	24,200	24,700	−500	−2.1
15	50.98	25,100	26,400	−1,300	−5.2

[a] Calculated using the least squares Equation 3 stated in the text (M_w = 4,907.4 P + 276,725).

where it can be shown that the denominator of Equation 9 is the weight fraction, $\frac{P}{100}$, of polymer considered in the numerator integral, and $M_0e^{.75\beta^2}$ is the weight average molecular weight, A, of the whole polymer. Hence, Equation 9 can be written in the forms shown by Equations 10.

$$\begin{aligned} M_w(P) &= \frac{100}{P} \frac{A}{\sqrt{\pi}} \int_{-\infty}^{z+\beta} e^{-z^2}\, dz \\ \frac{P}{100} &= \frac{1}{\sqrt{\pi}} \int_{-\infty}^{z+\beta/2} e^{-z^2}\, dz \end{aligned} \qquad (10)$$

Let erf z be defined by the error function shown in Equation 11.

$$\text{erf}\,(z) = \frac{2}{\sqrt{\pi}} \int_0^z e^{-z^2}\, dz \qquad (11)$$

This error function has been evaluated for various values of z. Adequate tables of this function appear in Peirce (*9*). Let antierf (z) be defined as the number whose erf is z, as in the case for log z and antilog z. Equation 10 may then be written as four simultaneous equations in terms of the weight average molecular weight of the whole polymer, A, and the weight average molecular weight of a high and low 10% fraction, H and L, respectively.

$$\begin{aligned} L &= 10A\,(0.5 \text{ erf } [\infty] + 0.5 \text{ erf } [z+\beta]) \\ 0.1 &= (0.5 \text{ erf } [\infty] + 0.5 \text{ erf } [z+\beta/2]) \\ H &= 10A\,(-0.5 \text{ erf } [z'+\beta] + 0.5 \text{ erf } [\infty]) \\ 0.9 &= (0.5 \text{ erf } [\infty] + 0.5 \text{ erf } [z'+\beta/2]) \end{aligned} \qquad (12)$$

Note that $\int_z^0 = -\int_0^z$ and $\int_{-\infty}^0 = -\text{ erf}\,(-\infty) = \text{erf}\,(\infty) = 1.$

Since erf $(\infty) = 1$, these equations may be arranged in the form shown by Equations 13.

$$\begin{aligned} \left(\frac{2L}{10A} - 1\right) &= \text{erf}\,(z+\beta) \\ (0.2 - 1) &= \text{erf}\,(z+\beta/2) \\ \left(1 - \frac{2H}{10A}\right) &= \text{erf}\,(z'+\beta) \\ (1.8 - 1) &= \text{erf}\,(z'+\beta/2) \end{aligned} \qquad (13)$$

In the ordinary Lansing-Kraemer distribution function, Equations 13 may be solved to yield the value of H in terms of A and L, noting the antierf (0.8) = 0.906.

Table III. Reliability of High 10% Fraction

	Low[a]	Av.[b]	High[c]	$\frac{L}{5A} - 1$[d]	Antierf, $\frac{L}{5A} - 1$	G[e]	F[f]	H[g], Calculated	R[h]	Comment
1	26,400	60,800	88,800	−0.9130	−1.210	0.602	0.3946	100,000	1.126	Poor
2	25,500	61,200	100,900	−0.9166	−1.224	0.588	0.4057	102,000	1.011	Excellent
3	36,300	69,900	107,000	−0.8964	−1.151	0.661	0.3499	100,000	0.935	Good
4	26,700	63,500	110,000	−0.9164	−1.224	0.588	0.4057	106,000	0.964	Excellent
5	33,100	70,700	114,000	−0.9064	−1.186	0.626	0.3760	110,000	0.965	Excellent
6	35,800	75,500	114,000	−0.9052	−1.181	0.631	0.3722	116,000	1.018	Excellent
7	35,700	80,600	116,000	−9.9114	−1.211	0.601	0.3954	131,000	1.129	Poor
8	34,000	79,900	119,000	−0.9148	−1.217	0.595	0.4001	132,000	1.109	Good
9	36,800	81,300	120,000	−0.9096	−1.197	0.615	0.3844	130,000	1.083	Good
10	31,600	66,000	120,000	−0.9044	−1.178	0.634	0.3699	102,000	0.850	Poor
11	36,700	81,500	123,000	−0.9102	−1.200	0.612	0.3868	132,000	1.073	Good
12	35,400	80,900	126,000	−0.9124	−1.208	0.604	0.3930	132,000	1.048	Excellent
13	36,800	76,300	128,000	−0.9036	−1.176	0.636	0.3684	116,000	1.094	Good
14	38,600	75,900	128,700	−0.8982	−1.157	0.655	0.3543	112,000	0.871	Poor
15	35,200	77,800	131,000	−0.9096	−1.197	0.615	0.3844	124,000	0.946	Excellent
16	36,300	82,300	133,000	−0.9116	−1.205	0.607	0.3907	133,000	1.000	Perfect
17	36,700	82,400	134,000	−0.9110	−1.203	0.609	0.3891	133,000	0.992	Excellent
18	29,100	69,000	134,000	−0.9156	−1.220	0.588	0.4057	116,000	0.865	Poor
19	33,800	78,800	134,000	−0.9142	−1.215	0.597	0.3985	130,000	0.970	Excellent
20	25,900	69,900	136,900	−0.9258	−1.263	0.549	0.4375	126,000	0.921	Good
21	34,100	87,000	138,000	−0.9216	−1.245	0.567	0.4226	150,000	1.087	Good
22	40,000	81,300	138,000	−0.9014	−1.168	0.644	0.3624	121,000	0.877	Poor
23	36,000	84,500	140,000	−0.9148	−1.217	0.595	0.4001	140,000	1.000	Perfect
24	26,600	80,200	142,000	−0.9338	−1.299	0.513	0.4681	156,000	1.099	Good
25	29,000	70,700	146,000	−0.9180	−1.238	0.582	0.4105	120,000	0.822	Repeat
26	31,500	79,100	153,000	−0.9212	−1.243	0.569	0.4210	138,000	0.902	Good
27	29,400	76,200	158,000	−0.9228	−1.250	0.569	0.4267	134,000	0.848	Poor
28	35,800	92,400	180,000	−0.9224	−1.248	0.564	0.4251	162,000	0.900	Good
29	31,500	89,300	180,000	−0.9294	−1.279	0.533	0.4510	168,000	0.933	Good
30	35,000	84,400	185,000	−0.9170	−1.226	0.586	0.4073	142,000	0.770	Repeat
31	19,100	97,000	223,000	−0.9610	−1.460	0.352	0.6186	247,000	1.108	Good

[a] Low, L, weight average molecular weight of low 10% fraction.

[b] Av., A, weight average molecular weight of whole clinical fraction.

[c] High, H, weight average molecular weight of high 10% fraction.

[d] The erf function is defined as:

$$\text{erf }(K) = \frac{2}{\sqrt{\pi}} \int_0^K \exp\,[-z^2]\,dz = I$$

such that,

$$K = \text{antierf }(I),$$

and erf $(-K) = -$erf (K).

[e] $G = 1.812 + \text{antierf}\left(\frac{L}{5A} - 1\right)$

[f] $F = 1 - \text{erf }(G)$

[g] H (calcd.) $= 4.15\ AF$

[h] $R = \frac{4.15\ AF}{H}$

$$H = 5A\left(1 - \text{erf}\left[1.812 + \text{antierf}\left\{\frac{2L}{10A} - 1\right\}\right]\right) \tag{14}$$

However, a better fit to the data was obtained if a constant multiplier, α, was used in the third equation of 12. Its purpose is to approximate the integration of this equation to a fixed, but finite, limit for the narrow range of molecular weights studied. This upper limit is constant for the dextran, since a top fraction is removed from the crude hydrolyzate each time in a precipitator under constant conditions of temperature, time, and precipitant concentration. The multiplication factor, α, may not be valid over a wider range of molecular weights. A more accurate correction term for this type of polymer would, of course, be a subtracted constant (erf $z'' + \beta$) to the right hand member of the third equation of 12.

However, the use of a single multiplier term, α, was found to be adequate over the molecular weight range from about 100,000 to 200,000. Its value was determined by averaging the values of H (obsd.)/H (calcd.), [using Equation 14 to evaluate H (calcd)] for the list of batches shown in Table III.

The constant multiplier, 5, in front of Equation 14 was then multiplied by α to yield a value of 4.15. Hence, the equation used in the calculations is shown by Equation 15.

$$H = 4.15A\left(1 - \text{erf}\left[1.812 + \text{antierf}\left\{\frac{2L}{10A} - 1\right\}\right]\right) \tag{15}$$

The results of this type of calculations are listed in Table III. The meaning of the functions used and the method of computation are listed in the footnotes to Table III. An error of 15% in H is to be expected at times owing to the accumulation of 5% in each of L, A, and H. Errors greater than this should encourage a repeat determination.

DISCUSSION

Although it has not been found possible to correlate molecular weight with methanol precipitation over the entire molecular weight range in order to verify light-scattering results, the authors feel that the scheme outlined above will prove adequate for routine investigations. It has not been possible to use the precipitation data in the high molecular weight region to get any reasonable correlation with molecular weight, even though the authors consider the volumetric data to be good to ±0.2% and the temperature control to be better than ±0.05° C. Attempts to correlate the weight average molecular weight of the whole clinical fraction with the volume per volume % of methanol required to precipitate 10.0, 20.0, 30.0, 40.0, and 50.0% of the clinical dextran have all failed. Also, correlation of amount of precipitation at a particular level of methanol—e.g., 85 volume per volume %—with weight average molecular weight of the whole fraction was poor.

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Received for review December 28, 1953. **Accepted** November 8, 1954.

Oxyhydrogen Burner for Determination of Sulfur in Drip Oils

LAWRENCE GRANATELLI

Pan American Refining Corp., Texas City, Tex.

Difficulties have been encountered in the determination of sulfur in drip oils when conventional methods of combustion are employed. This investigation was undertaken to determine the feasibility of using an oxyhydrogen burner as a means of burning the sample. The apparatus described utilizes the oxyhydrogen burner of the Beckman flame photometer. The sample is aspirated through the burner capillary tube directly into the flame; products of combustion are absorbed in hydrogen peroxide, and sulfur is determined gravimetrically as barium sulfate. Under the conditions employed, a burning rate of about 24 ml. per hour is maintained. This rapid burning rate favors the analysis of samples containing low concentrations of sulfur, as large samples can be burned in a relatively short time. The apparatus is suited to the determination of sulfur in a variety of petroleum liquids. Samples of drip oil and of paraffinic naphtha of known sulfur content were studied. Analysis of samples containing from 0.006 to 0.112% sulfur showed standard deviations of ±0.001 to ±0.004%. Excellent recovery obtained on a solution of elemental sulfur indicates that the method is suited for the determination of total sulfur. With minor modification the method may be extended to the determination of halogens in a variety of petroleum samples.

THE determination of small concentrations of sulfur in drip oils by conventional methods of burning the sample has proved unsatisfactory in this laboratory. A drip oil may be defined as a gasoline boiling range hydrocarbon oil recovered as a by-product from the cracking of petroleum gases. Burning the oil by the lamp method (*2*) results in a smoky flame and resin formation on the wick. These difficulties are attributed to high concentrations of aromatics and diolefins in these oils. Neither dilution with 2-propanol (*5*) nor use of modified burners (*4, 6*) appreciably altered the burning characteristics of these oils. The oxygen bomb method (*3*) proved unsatisfactory, owing to limited sample size, volatility of the sample, and the frequency of incomplete combustions. Flashing of volatile components was the main impediment to satisfactory operation in the quartz tube method (*1*).

This paper presents the results of an investigation whereby difficulties encountered in obtaining complete combustion of drip oils by conventional methods have been circumvented by burning the sample in the flame of an oxyhydrogen burner. The drip oil is aspirated through the burner directly into the oxyhydrogen flame. Sulfur oxides formed are absorbed and oxidized to sulfuric acid in hydrogen peroxide solution and determined gravimetrically as barium sulfate.

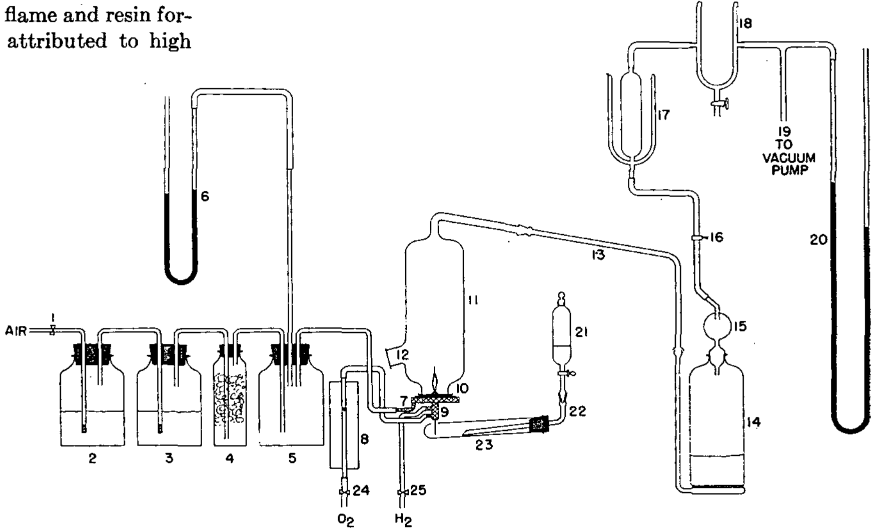

Figure 1. Assembly for burning liquid petroleum samples

1. Needle valve
2. Sodium hydroxide scrubber
3. Hydrogen peroxide–sulfuric acid scrubber
4. Spray trap
5. Empty chamber
6. Mercury manometer
7. Air inlet chamber
8. Flowmeter
9. Oxyhydrogen burner
10. Rubber gasket
11. Chimney
12. Blow-out port
13. Connecting arm
14. Absorber
15. Spray trap
16. Screw clamp
17. Knock-back
18. Condenser
19. Vacuum pump
20. Mercury manometer
21. Weighing buret
22. Delivery tube
23. Test tube
24. Needle valve
25. Needle valve

Lane (*5*) has shown that the lamp method cannot be used for quantitative determination of elemental sulfur or for total sulfur unless elemental sulfur is known to be absent. During the course of this investigation the utility of the oxyhydrogen burner technique for determining total sulfur was studied. The analysis of a solution of elemental sulfur by this method indicates that the apparatus is applicable for the determination of elemental sulfur in hydrocarbon mixtures.

REAGENTS

Hydrogen peroxide, 3%; dilute 30% hydrogen peroxide (Fisher certified reagent) with distilled water.

2-Ethylhexanol.

Hydrochloric acid, C.P., specific gravity 1.18.

Barium chloride, C.P., 10% solution in distilled water.

Sodium hydroxide, C.P., 10% in distilled water.

Hydrogen peroxide–sulfuric acid solution; add 2 ml. of concentrated sulfuric acid to 300 ml. of 3% hydrogen peroxide.

Hydrogen, Airco.

Oxygen, Linde.

APPARATUS

Assembly of the apparatus is shown schematically in Figure 1.

Air Purification System. Scrubber 2 contains 300 to 400 ml. of 10% sodium hydroxide. Scrubber 3 contains the same amount of hydrogen peroxide–sulfuric acid solution (3% hydrogen peroxide–1% sulfuric acid). Bottle 4 is packed with glass wool to serve as a spray trap. Bottle 5 is assembled empty and connected to mercury manometer, 6, and air inlet chamber, 7. Absorbents in the system are renewed twice weekly. The rate of flow of compressed air (at a pressure of about 6 pounds per square inch gage) through the purification train is regulated by needle valve 1.

Burner, Chimney Assembly. Oxygen and hydrogen gas cylinders are fitted with pressure reducer gages. The oxygen cylinder is connected, through a flow meter, 8, to the oxygen inlet tube of the burner, 9 (burner-atomizer, hydrogen-oxygen, Serial 9240, Beckman Instruments, Inc., South Pasadena, Calif.). The hydrogen cylinder is connected to the hydrogen inlet tube of the burner.

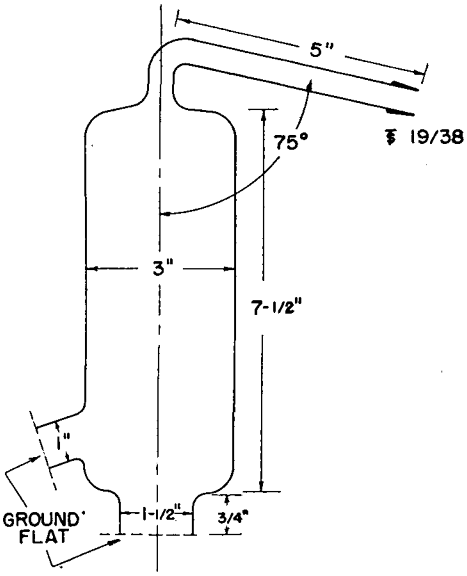

Figure 2. Borosilicate glass chimney

An air-tight seal between chimney 11 and burner is made by means of a rubber gasket, 10 (crucible holder, Sargent, soft, pliable rubber), which rests on the air inlet chamber, 7. The chimney is provided with a blow-out port, 12, to which a thin microscope cover glass is sealed with Fisher's Sealit. Connection between chimney and absorber is made by means of a borosilicate glass tube, 13, fitted with a $^{19}/_{38}$ ₮ outer joint and an $^{18}/_{9}$ semiball joint. A more detailed view of the chimney is shown in Figure 2. The air inlet chamber is shown in detail in Figure 3.

Absorber. A gas washing bottle, 14, with coarse fritted-glass disk is used for the absorber. An $^{18}/_{9}$ semiball joint is fused to the inlet tube of the absorber. The absorber is fitted with a spray trap, 15, and is connected to a knock-back by heavy rubber tubing. A screw clamp, 16, is placed between spray trap and knock-back. Condenser 18 follows the knock-back and is connected to vacuum pump 19 and mercury manometer 20.

Sample Assembly. The sample to be burned is contained in a 60-ml. weighing buret, 21 (Friedman and LaMer), which is fitted into a delivery tube, 22, having a $^{7}/_{25}$ ₮ outer joint. The delivery tube extends through a rubber stopper into a test tube, 23, having a 2-mm. hole drilled near the bottom end. The capillary tube of the burner extends through the hole in the test tube into the sample to be burned.

PROCEDURE

The specific pressures and flow rates cited in the following detailed description of operating the apparatus for burning drip oils do not apply generally but depend on characteristics of individual burners.

The absorber, knock-back, and condenser are cooled with ice-water mixture. The absorber is charged with 100 ml. of 3% hydrogen peroxide. One milliliter of 2-ethylhexanol is added to minimize frothing. A 6.3-mil (34 B and S gage) Nichrome wire is inserted through the burner capillary tube and up to, but not beyond, the burner tip. Oxygen and hydrogen pressures at the gas cylinders are regulated to 10 and 4 pounds per square inch gage, respectively. The oxygen flow rate to the burner is adjusted to about 2 liters per minute. Hydrogen is allowed to flow to the burner and the gas mixture is ignited. The hydrogen needle valve is regulated to yield a flame $^{3}/_{4}$ to 1 inch in height. Compressed air is turned on. The vacuum pump is started and the lighted burner is inserted into the chimney. While maintaining the manometer reading at the air scrubber train at about 5 mm. of mercury below atmospheric pressure by means of needle valve 1 (Figure 1), the manometer reading at the scrubber system is adjusted to 75 to 80 mm. of mercury below atmospheric by means of screw clamp 16. The system is checked for leaks by turning the air flow off. If no leaks are present the manometer on the air scrubber train should indicate 50 to 60 mm. of mercury below atmospheric pressure. Air flow through the apparatus is resumed; the system is now ready for burning the sample.

The sample to be burned is weighed in the 60-ml. weighing buret and placed in the sample assembly. A few milliliters of sample are admitted to the test tube. The assembly is raised so that the burner capillary tube extends through the hole in the test tube into the sample.

When most of the sample in the test tube is burned, it is replenished with more from the buret. This process is continued until all the sample is transferred from the buret to the test tube. To ensure quantitative transfer of the sample, two 1-ml. portions of 2-propanol are used to wash down the buret wall. These washings are added in small increments to the test tube and burned.

When burning has been completed, the sample assembly is lowered; the hydrogen and then the oxygen supplies to the burner are turned off; the burner is removed from the chimney; and the vacuum is discontinued.

The knock-back and spray trap are rinsed with distilled water which is allowed to flow into the absorber. The absorber solution is quantitatively transferred to a 400-ml. beaker. The chimney and connecting tube are rinsed with hot distilled water and the washings are added to the beaker. Two milliliters of concentrated hydrochloric acid are added to the solution and the beaker is placed on a hot plate. When the solution is at or near the boiling point, 10 ml. of 10% barium chloride are added. The mixture is evaporated to about 100 ml. and the precipitate is allowed to stand for several hours before filtration. The barium sulfate precipitate is determined gravimetrically. Blank sulfur determinations on all reagents used are subtracted from the final analysis.

EXPERIMENTAL RESULTS

To evaluate the apparatus and the accuracy of the method, solutions containing known amounts of sulfur (as thiophene) in paraffinic naphtha and in drip oil were prepared and analyzed. The results of these analyses are shown in Tables I and II. Blank sulfur determinations on base stocks were subtracted from the analyses. The thiophene used (Eastman Kodak Co.) was analyzed by the oxygen bomb method (*3*) and found to have a purity (as sulfur) of 97.5% (average of three determinations). To determine the utility of this method for the determination of total sulfur, a solution of elemental sulfur (monoclinic) in benzene was analyzed. Table III gives the results of this analysis. The monoclinic sulfur was obtained by the recrystallization of commercial sulfur from carbon disulfide and finally from pyridine.

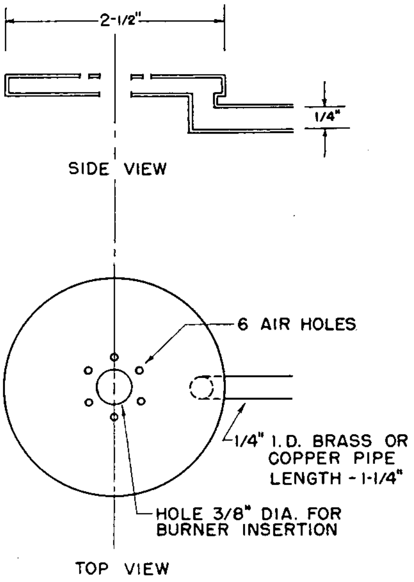

Figure 3. Air inlet chamber

Table I. Determination of Sulfur[a] in Paraffinic Naphtha

No.	Sample Size, Ml.[b]	Sulfur, % Added	Found	Dev. from mean
1	25	0.108	0.109	+0.001
2	25		0.110	+0.002
3	20		0.109	+0.001
4	20		0.110	+0.002
5	15		0.107	−0.001
6	15		0.112	+0.004
7	10		0.108	0.000
8	10		0.102	−0.006
9	5		0.102	−0.006
10	5		0.105	−0.003
11	5		0.112	+0.004
12	5		0.105	−0.003
		Mean	0.108	Std dev. ±0.0035
13	25	0.052	0.051	−0.002
14	25		0.052	−0.001
15	20		0.052	−0.001
16	20		0.052	−0.001
17	15		0.053	0.000
18	15		0.054	+0.001
19	15		0.051	−0.002
20	15		0.054	+0.001
21	10		0.055	+0.002
22	10		0.056	+0.003
		Mean	0.053	Std. dev. ±0.0017
23	50	0.006	0.006	−0.002
24	40		0.007	−0.001
25	30		0.007	−0.001
26	30		0.008	0.000
27	20		0.010	+0.002
28	20		0.012	+0.004
		Mean	0.008	Std. dev. ±0.0030

[a] Added in form of thiophene.
[b] Density, 0.76 gram/ml.

Table II. Determination of Sulfur[a] in Drip Oil

No.	Sample Size, Ml.[b]	Sulfur, % Added	Found	Dev. from mean
1	25	0.112	0.113	0.000
2	25		0.114	+0.001
3	20		0.114	+0.001
4	20		0.113	0.000
5	15		0.113	0.000
6	15		0.114	+0.001
7	10		0.111	−0.002
8	10		0.113	0.000
9	5		0.110	−0.003
10	5		0.112	−0.001
		Mean	0.113	Std. dev. ±0.0013
11	25	0.058	0.058	−0.001
12	25		0.058	−0.001
13	20		0.059	0.000
14	20		0.059	0.000
15	20		0.060	+0.001
16	15		0.058	−0.001
17	15		0.060	+0.001
18	15		0.062	+0.003
19	10		0.058	−0.001
20	10		0.060	+0.001
		Mean	0.059	Std. dev. ±0.0013

[a] Added in form of thiophene.
[b] Density, 0.86 gram/ml.

Oxidation by the quartz tube method (*1*) and gravimetric analysis indicated a purity of 100 ± 0.2%.

Examination of Tables I and II reveals that standard deviations of ±0.001 to 0.004% sulfur were experienced in the determination of sulfur in petroleum samples containing from 0.006 to 0.112% sulfur. A study of the effect of sample size on the accuracy of analysis indicates that the larger samples minimize errors which are inherent in the procedure. The excellent recovery shown in Table III for the determination of elemental sulfur indicates that the apparatus is suited for total recovery of sulfur (both elemental and combined).

DISCUSSION

By maintaining gas flow rates and pressure differentials constant, wires of various diameters and lengths inserted in the burner capillary tube may be employed to regulate the rate of flow of sample into the oxyhydrogen flame. Too rapid a sample flow causes the flame to be extinguished. The burner with a full length of 6.3-mil wire in the capillary tube was used to burn drip oils at a rate of about 24 ml. per hour. By reducing the length of wire in the capillary, liquids such as naphtha, benzene, and iso-octane were burned at a more rapid rate. 2-Propanol was burned using the full aperture of the burner capillary. Crude oil has been burned by further reducing the capillary aperture with a 10-mil wire, whereas for burning heavy gas oil, resort to dilution with benzene was necessary.

Another means of controlling the rate of sample flow consists of regulating the pressure differential in the system. Reducing the flow of air to the chimney increases the rate of flow of sample to the flame. Iso-octane and 2-propanol may be burned continuously with no air flowing through the system. With samples of higher heat of combustion, air flow is necessary to keep the flame from being extinguished. A rapid air flow serves also to minimize the temperature of the chimney around the burner.

During this investigation, chimneys of various sizes have been used. These ranged in dimensions from 2 inches in diameter by 5 inches in length to 3.5 by 14 inches. The chimney described here (Figure 2) has been found suitable for burning drip oils and most other liquids. Smaller chimneys may be used for liquids such as iso-octane or benzene but will not function satisfactorily for drip oils. Since sulfuric acid is adsorbed on the chimney surface, it is desirable to employ a chimney of minimum surface area.

By burning concentrated solutions of thiophene in iso-octane it was learned that about 2% of the sulfur in the sample is adsorbed on the chimney and side-arm walls as sulfuric acid. By placing an absorber containing water before the hydrogen peroxide absorber, it was learned that about 9% of the sulfur in the sample is oxidized directly to sulfuric acid before coming in contact with hydrogen peroxide. Because of the adsorption of sulfuric acid on the glass walls, hot distilled water was used to rinse the chimney and side arm.

Gravimetric analysis, rather than alkalimetric titration, was used since considerable nitric acid is formed by oxidation of atmospheric nitrogen during the process of burning the sample. This was shown by operating the apparatus without sample and with the capillary aperture closed for a period of 2 hours. The nitric acid which was formed was titrated with alkali and found to be equivalent to about 0.03% sulfur in a 20-gram sample. Improvement in the accuracy of analysis of samples containing less than 0.01% sulfur may be accomplished by using a turbidimetric procedure. An alkalimetric procedure may be employed if the sample is burned in a nitrogen-free atmosphere and if no other acid-forming elements are present in the sample. For the purpose of evaluating this combustion technique the use of gravimetric analysis was found both convenient and practical.

The large amount of heat formed by burning the sample is dissipated in the ice-water bath in which the absorber is placed. In the absence of this cooling bath, considerable water collects in the knock-back and condenser. Furthermore, the water vapor collected in the calcium chloride absorbers between condenser and vacuum pump would necessitate more frequent refilling of these absorbers and involve possible damage to the pump.

The provision of the blow-out port in the chimney has served its purpose, especially during the preliminary investigation of the

Table III. Determination of Elemental Sulfur in Benzene

No.	Sample Size, Ml.	Sulfur, % Added	Found	Dev. from mean
1	25	0.144	0.146	+0.001
2	25		0.145	0.000
3	25		0.144	−0.001
4	20		0.146	+0.001
5	15		0.145	0.000
6	10		0.144	−0.001
7	10		0.143	−0.002
		Mean	0.145	Std. dev. ±0.0011

apparatus. Only two ruptures of the port have occurred during a period of burning more than 350 samples. No harm to the operator or damage to the equipment was experienced. In the author's opinion, rupture of the port will occur only if the vacuum pump fails to operate or if the flame is extinguished and the chimney fills with combustible gases before the sample assembly is lowered. For these reasons it is recommended that the operator attend the apparatus throughout the time needed to burn the sample.

Operating the apparatus under the conditions cited in the procedure, the rubber gasket will function properly for 7 to 8 days. If the rate of sample flow is increased appreciably the gasket may need to be replaced daily. Under more severe burning conditions a gasket per sample may be required. Examination of the per cent deviation column in Tables I and II shows that the majority of the deviations are positive with respect to the amount of sulfur added. This is attributed to the presence of sulfur in the rubber gasket. Gravimetric analysis of sulfur in the rubber gasket indicated more than 2% present.

Since the conclusion of the work reported here the apparatus has been modified by sealing the burner in a $^{45}/_{50}$ ₸ outer glass joint and connecting it to the chimney through a $^{45}/_{50}$ ₸ inner joint. This modification not only improved the repeatability of the results but eliminated the possibility of sulfur contamination from a rubber seal. The sample of drip oil containing 0.058% sulfur (Table II) showed deviations from 0.000 to 0.004% with the rubber gasket and 0.000 to 0.001% with the ₸ glass joint.

The extension of the method to the determination of halogens in petroleum liquids is obvious. Limited work has been done on the determination of organically bound chlorine in drip oils. Results are comparable to those reported for sulfur.

ACKNOWLEDGMENT

The author wishes to express his thanks to J. A. Grant for suggesting the problem, to R. L. Aiken for designing the air inlet chamber and offering helpful suggestions throughout the course of the investigation, to K. C. Nichols and Mildred Gibson for operating the apparatus, and to Pan American Refining Corp. for permission to publish this work.

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RECEIVED for review August 16, 1954. Accepted November 8, 1954.

Indirect Polarographic Determination of Propylenediamine

A. D. HORTON, P. F. THOMASON, and M. T. KELLEY
Analytical Chemistry Division, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Propylenediamine has been determined indirectly by polarographic estimation of the propylenediamine-copper complex in a supporting electrolyte of 0.1*M* potassium chloride. Propylenediamine in concentrations of 26 to 97 γ per ml. can be determined with an over-all relative standard deviation of 0.70%. The method is recommended for the estimation of microgram amounts of propylenediamine or other chelating amines in solutions that do not contain other substances that will form complexes with copper or that will reduce at half-wave potentials within 0.2 volt of the half-wave potential of the propylenediamine-copper complex, which varies slightly with variation in propylenediamine concentration.

POLAROGRAPHIC studies of the propylenediamine-copper complex have been made by Calvin and Bailes (*1*) to determine the stability of chelate compounds and by Carlson and coworkers (*2*) to determine the equilibrium constant of amine complexes with copper ions. In these studies copper was added in small amounts to amounts of propylenediamine that were in stoichiometric excess of the copper. In such cases, a single polarographic wave was obtained and the diffusion current was proportional to the amount of copper present in the solution.

It was proposed to reverse these conditions—that is, to add samples of propylenediamine to copper chloride solutions in which the total amount of copper was fixed at a constant amount that would always be in stoichiometric excess of the propylenediamine to be determined. A noncomplex-forming supporting electrolyte was needed to make the conditions suitable for the polarographic determination of the propylenediamine-copper complex. The polarogram of such a solution was expected to show a double wave analogous to that obtained when a copper-ammonia complex is determined polarographically (*4*). Subsequent investigations have shown that the double wave obtained was not a split wave that resulted from the stepwise reduction of copper, but instead was an initial wave for excess copper and a second wave for the propylenediamine-copper complex.

REAGENTS AND APPARATUS

All the reagents used, except propylenediamine, were reagent grade.

Copper chloride solution, 500 γ of copper per ml. Prepare by placing 0.1342 gram of cupric chloride dihydrate in a 100-ml. volumetric flask and diluting to volume with double-distilled water.

Potassium chloride solution, 1*M*. Prepare in double-distilled water.

Propylenediamine, 880 γ per ml. purified by azeotropic distillation with toluene. A dilution of the azeotrope was made with double-distilled water and the solution was standardized by titration with standard hydrochloric acid solution to the methyl red end point.

Polarograph, ORNL high sensitivity (*3*). The dropping mercury electrode (D.M.E.) was used as the indicating electrode and the saturated calomel electrode (S.C.E.) as the reference electrode.

EXPERIMENTAL

All polarographic analyses were carried out in an air-conditioned laboratory where the temperature was controlled to within ±2° F.

In the evaluation of this indirect polarographic method for the determination of propylenediamine, it was desired to find a supporting electrolyte that would not complex either propylenediamine or copper. A sodium borate buffer of pH 9.0 was tried first. The wave of the propylenediamine-copper complex (second wave) was well defined in this medium, but the diffusion current decreased after the solution had remained in the polarographic cell for a short time. The diffusion current decrease was due to the gradual change in the equilibrium of the reaction between copper and propylenediamine. Carlson and coworkers (*2*) have found that copper forms a stable complex with propylenediamine, ethylenediamine, and other chelating amines. They have suggested that the formula for the propylenediamine-copper complex is $Cupn_2Cl_2$. However, this complex prevails only when propylenediamine is in stoichiometric excess of the copper. When copper is in excess, the complex $CupnCl_2$ prevails. In either case, $Cupn_2Cl_2$ is formed first. If copper is in excess, the $Cupn_2Cl_2$ decomposes into $CupnCl_2$.

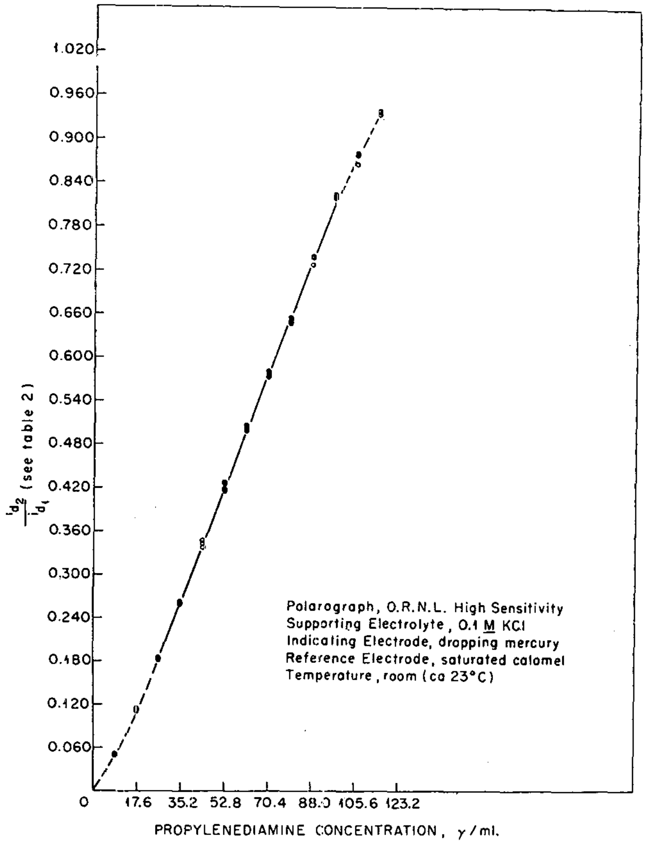

Figure 1. Calibration curve for polarographic determination of propylenediamine (pn) as the pn-Cu complex

Table I. Polarographic Determination of Propylenediamine as the pn-Copper Complex

(Supporting electrolyte, 0.1*M* KCl. Diffusion current of 50 γ Cu per ml. = 4.67 μa. (id_1).)

Known Concentration pn, γ/Ml.	Diffusion Current pn-Cu Complex (id_2), μA	$\frac{id_2}{id_1}$	pn Found, γ/Ml.	Rel. Std. Deviation
26.4	0.850	0.182	26.26	0.80
	0.865	0.185	26.68	
	0.850	0.182	26.26	
	0.860	0.184	26.54	
35.2	1.210	0.259	34.93	0.54
	1.225	0.262	35.33	
	1.225	0.262	35.33	
	1.220	0.261	35.20	
61.6	2.350	0.503	61.60	0.47
	2.365	0.506	61.96	
	2.350	0.503	61.60	
	2.335	0.500	61.23	
79.2	3.04	0.651	79.20	0.48
	3.02	0.647	78.71	
	3.05	0.653	79.44	
	3.03	0.649	78.96	
	3.06	0.655	79.68	
96.8	3.84	0.822	96.68	0.48
	3.84	0.822	96.68	
	3.87	0.829	97.51	
	3.83	0.820	96.45	
105.6	4.10	0.878	106.1	0.94
	4.11	0.880	106.3	
	4.04	0.865	104.5	
	4.04	0.865	104.5	
114.4	4.36	0.934	113.7	0.37
	4.40	0.942	114.6	
	4.40	0.942	114.6	
	4.39	0.940	114.4	

Calvin and Bailes (*1*) used both potassium chloride and potassium nitrate as noncomplexing electrolytes to obtain polarograms of solutions of the order of $10^{-3}M$ in propylenediamine that contained copper in great excess of the propylenediamine. They used a 50 volume % mixture of pyridine and water rather than pure water as a solvent for the complex because the dissolution of mercury that occurs at potentials below the order of −0.1 volt is less in the pyridine-water mixture than in water. However, the dissolution of mercury did not interfere either with the formation of the complex wave or with the formation of the copper wave when no propylenediamine was present, so water was chosen as the solvent for the sample.

Polarograms were obtained of a series of solutions of propylenediamine-copper complexes by use of potassium chloride as the supporting electrolyte. The diffusion currents of these waves were related to that of the copper reduction wave by obtaining the ratio of the diffusion current of the wave for the complex to that of the wave for pure copper. The maximum concentration that could be determined by the method is limited by the concentration of propylenediamine that would be equivalent to 50 γ of copper per ml. The range could have been extended to the limit of sensitivity of the polarographic by increasing the copper concentration.

These experiments were repeated with potassium nitrate as the supporting electrolyte, but the diffusion currents of the complex waves were not as reproducible, as were those with potassium chloride as the supporting electrolyte.

PROCEDURE

Analysis of a Sample. Determine the diffusion current of a solution that contains 50 γ of copper per ml. of 0.1*M* potassium chloride solution. Pipet 1.00 ml. of a solution that contains 500 γ of copper, as cupric chloride, per ml. into a 10-ml. volumetric flask. Pipet into the flask a volume of sample (not more than 7 ml.) that will give a concentration of 26 to 97 γ of propylenediamine per ml. when the solution is diluted to 10 ml. Add 1.0 ml. of 1.0*M* potassium chloride solution to the flask.

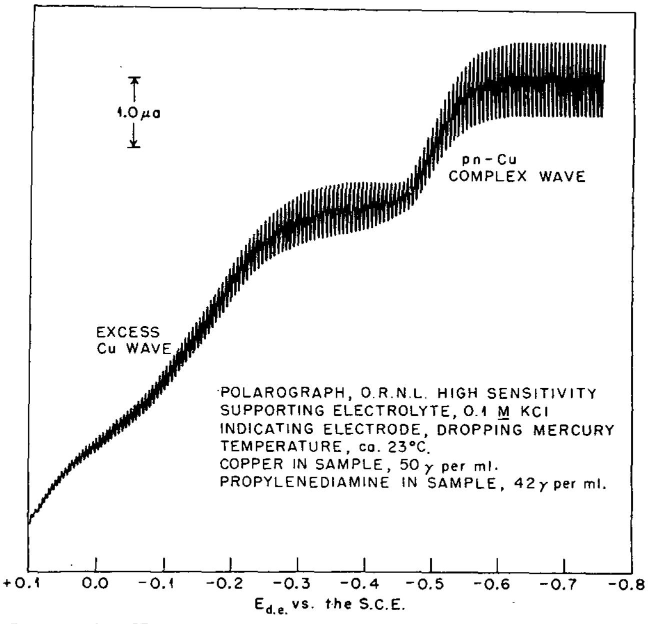

Figure 2. Typical polarogram of the pn-Cu complex

Add 0.1 ml. of 1.0% gelatin solution, dilute the solution to 10 ml., and mix it thoroughly. Pipet a suitable aliquot of the final solution into the polarographic cell, and determine the diffusion current of the wave of the propylenediamine-copper complex, which is the second section of the double wave. Calculate the ratio of the diffusion current of the complex wave to the diffusion current of the solution of 50 γ of copper per ml. of 0.1M potassium chloride. From the calibration curve, determine the propylenediamine concentration in micrograms per milliliter that corresponds to the ratio calculated in the previous step.

The 10-ml. sample volume given above is suggested as a convenient volume, but the range of the method may be extended by use of a much smaller volume, bearing in mind the recommended concentrations of copper, supporting electrolyte, and gelatin.

Preparation of Calibration Curve. Prepare the calibration curve as indicated above, by use of a standardized propylenediamine solution instead of the sample, and repeat the procedure for a reasonable number of values within the concentration range indicated above.

RESULTS AND DISCUSSION

Figure 1 shows a plot of the ratios of the diffusion currents of the propylenediamine-copper complex waves to the diffusion current of a solution of 50 γ of copper per ml. on the ordinate *vs.* concentration of propylenediamine on the abscissa. The minimum propylenediamine concentration that would produce a well-defined wave was 8.8 γ per ml., and the maximum concentration that could be determined was 114.4 γ per ml. The portion of the graph between the concentrations of 26.4 and 96.8 γ per ml. is linear. In the lower concentration range, maxima tended to form, but they were eliminated by the addition of gelatin in the amount of 0.01% by volume. Table I gives the results obtained for solutions of known concentration that were prepared from the standardized propylenediamine solution described above. The results obtained within this range have an over-all precision of 0.70%. The value for over-all precision was obtained by the formula

$$\text{Over-all precision} = \sqrt{\frac{\Sigma_0^n(nS^2)}{(\text{total } n) - N}}$$

where N represents the number of sets of data, n represents the number of determinations in each set, and S represents the relative standard deviation for each set of data.

An investigation of the suitability of this method for the determination of micro amounts of propylenediamine in solutions or soluble solids has shown the reason for a double wave. The two waves, observed in a solution containing a concentration of cupric ion exceeding that of the propylenediamine (Figure 2), are due to the two-electron reduction of the uncomplexed cupric ion to metal (wave I) and the two-electron reduction of the cupric monoamine complex to the metal (wave II). Figure 2 shows a typical polarogram of the propylenediamine-copper complex in excess copper solution.

The approximate half-wave potentials ($E_{1/2}$) of the propylenediamine-copper complex wave vary from −0.4 to −0.6 volt *vs.* S.C.E. with variations in propylenediamine concentration of the sample. The more positive half-wave potential is obtained with the minimum propylenediamine concentration in the range of this method, and the more negative value is obtained with the maximum concentration.

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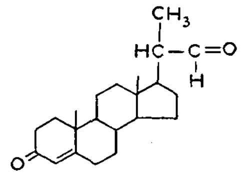
RECEIVED for review June 10, 1954. Accepted November 3, 1954.

Use of Sodium Borohydride for Determination of 3-Ketobisnor-4-cholene-22-al

ERIK H. JENSEN and WILLIAM A. STRUCK
Research Laboratories, The Upjohn Co., Kalamazoo, Mich.

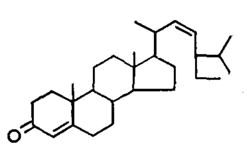
This investigation was undertaken to devise a method for determining the purity of 3-ketobisnor-4-cholene-22-al. This aldehyde is an important intermediate in a partial synthesis of progesterone from either ergosterol or stigmasterol. Accordingly, the determination of its purity is important. The essential reaction is a sodium borohydride reduction in alkaline medium, and the purity of 3-ketobisnor-4-cholene-22-al is based on the amount of borohydride used in the reduction of the aldehyde group. The accuracy of the method is excellent and the standard deviation is ±0.34% for samples in the 80 to 100% purity range.

TWO methods of obtaining progesterone from 4,22-stigmastadien-3-one have been reported by Heyl and Herr (*2*, *3*, *4*). In both of these methods the key intermediate is 3-ketobisnor-4-cholene-22-al-(I).

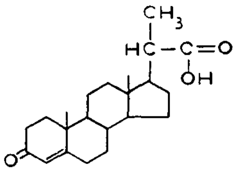


(I)

This aldehyde is prepared from 4,22-stigmastadien-3-one(II) and is contaminated principally with (II) and 3-ketobisnor-4-cholenic acid(III), the oxidation product of the aldehyde. Other undefined contaminants may be present.

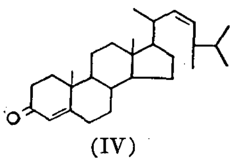


(II)



(III)

Similarly, Sheperd *et al.* (*10*) have described the synthesis of progesterone from ergosterol. Again, the 3-ketobisnor-4-cholene-22-al (I) is a key intermediate. In this instance, the aldehyde is prepared by ozonolysis of the side-chain double bond of 4,22-ergostadien-3-one (IV).



(IV)

In view of these facts a suitable assay for the determination of the purity of 3-ketobisnor-4-cholene-22-al was desired.

The conventional methods (*1, 5, 7, 8, 9, 11*) of determining carbonyl function do not distinguish sufficiently between aldehyde and keto groups in high molecular weight compounds. In this case, the problem is particularly complex. Both an aldehyde and a keto group are present in the compound in question and the known impurities all contain one keto group.

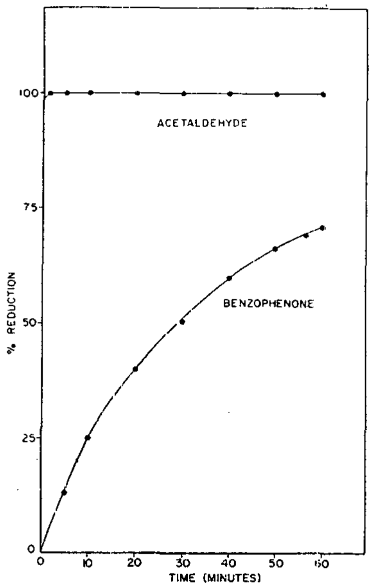
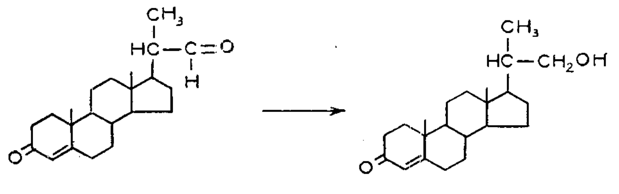


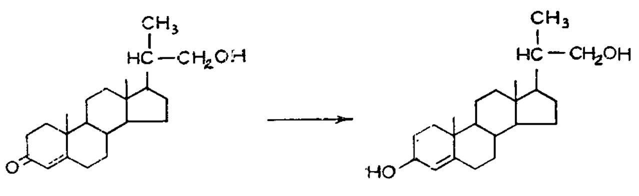
Figure 1. Rate of reduction of an aldehyde and a ketone

In studying the reducing properties of sodium borohydride toward various aldehydes and ketones, it was observed that there is in general a profound difference between the rates of reduction of aldehydes and ketones. Generally speaking, aldehydes are reduced almost instantaneously in alkaline solution, whereas ketones are reduced at a much slower rate (Figure 1).

Furthermore, the first part of the ketone reduction curve is almost a straight line. In the case of the 3-ketobisnor-4-cholene-22-al, the net titration curve is as shown in Figure 2. The vertical part of the curve represents the almost instantaneous reduction of the aldehyde group:



The sloped portion of the curve indicates the partial reduction of the 3-keto group:



Thus, the intercept of the straight-line portion of the curve with the zero time axis gives a measure of the content of aldehyde.

All of the data reported are based on the measurement of the consumption of sodium borohydride. The unreacted excess sodium borohydride is determined by the iodometric method of Lyttle, Jensen, and Struck (*6*).

PROCEDURES

Reagents.

Potassium iodate, 0.25*N*, standard solution.

Sodium thiosulfate, 0.10*N*, standard solution.

Sodium borohydride, 0.5*N* in 1.0*N* sodium hydroxide, freshly prepared from commercial material purified as follows: Fifty grams of sodium borohydride and 1 gram of active carbon (Darco G-60) are heated in 75 ml. of 1.0*N* sodium hydroxide at 33° to 36°C. until the sodium borohydride has dissolved. The solution is filtered by gravity through a medium-coarse fritted glass filter maintained at 35° C. and the filtrate is allowed to stand in a covered crystallizing dish for 24 hours at refrigerator temperature (4° C.). The crystals are collected on a medium-coarse fritted glass filter, dried in a desiccator, and finally dried to constant weight at 60° to 80° C. and 0.1 to 0.2 mm. of mercury. The purity of the product is 98 to 99%.

Sulfuric acid, 4*N*.

Potassium iodide, reagent grade.

Amylose starch indicator (G. F. Smith Chemical Co.).

Dioxane, technical grade, redistilled over sodium hydroxide and sodium borohydride and stored over sodium.

General Procedure for Obtaining Rate Curves. A five-gram sample of steroid, accurately weighed, is placed in a 100-ml. volumetric flask. The sample is dissolved and diluted to volume with dioxane. A 10-ml. aliquot is withdrawn and delivered into a 250-ml. iodine flask. Two milliliters of water are added, followed immediately by exactly 5.00 ml. of the sodium borohydride solution. Since accurate measurement of the reaction time is essential, a stop watch is started at the instant the pipet delivering the sodium borohydride is half empty. After the sodium borohydride has been added, the flask is stoppered and swirled and allowed to stand for a predetermined time interval. At the end of this reaction time, 20.00 ml. of 0.25*N* potassium iodate are added, followed rapidly by 2 grams of potassium iodide and 10 ml. of 4*N* sulfuric acid. At the instant the acid is added, the stop watch is stopped and the time recorded. The liberated iodine is then titrated with 0.10*N* sodium thiosulfate using amylose starch indicator. Subsequent aliquots of the steroid solution are treated in exactly the same way, choosing reaction times that give a descriptive rate curve. Ordinarily six to eight aliquots covering the time range from 2 to 60 minutes give enough points for an accurate picture of the course of the reaction. A blank is run on the solvents.

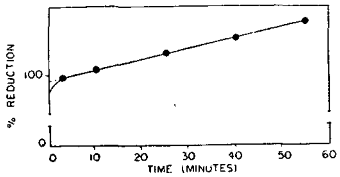


Figure 2. Rate of reduction of 3-ketobisnor-4-cholene-22-al

Calculation. The results are plotted in a coordinate system with time as the abscissa and percentage of reduction as the ordinate.

$$\%\ \text{reduction} = \frac{(b - a) \times N \times \text{mol. wt.}}{p \times 20}$$

where p = sample weight in grams in a 10-ml. aliquot
a = milliliters of sodium thiosulfate used for blank
b = milliliters of sodium thiosulfate used for sample
N = normality of sodium thiosulfate
Mol. wt. = molecular weight of compound

Specific Procedure for Determining Purity of 3-Ketobisnor-4-cholene-22-al. A sample of 1.0 to 1.2 grams is weighed accurately into a 25-ml. volumetric flask, dissolved, and diluted to volume with dioxane. A 10-ml. aliquot of this solution is pipetted into a 250-ml. iodine flask containing exactly 5.00 ml. of the

Table I. Recovery of Known Increments of 3-Ketobisnor-4-cholene-22-al in 4,22-Stigmastadien-3-one

Known Compn. of Mixt., % Aldehyde Added	% Aldehyde Found
90.15	90.2
50.02	49.8
29.03	29.1
2.61	3.0

sodium borohydride solution plus 2.0 ml. of water. The stop watch is started when the pipet delivering the steroid solution is half empty. From this point on the procedure is identical with the general method, except that the reaction time for this aliquot should be 5 minutes. A second 10-ml. aliquot of the sample is run in the same way; the reaction is allowed to proceed for 10 minutes. A blank must accompany each determination.

Calculation. Each determination is calculated from the given formula. The results of the two determinations are plotted with time as the abscissa and the results in per cent as the ordinate. A straight line is drawn through these points intersecting the ordinate. This zero time intercept gives directly the per cent of 3-ketobisnor-4-cholene-22-al in the sample.

RESULTS AND DISCUSSION

Titration curves for the following compounds were obtained: progesterone, 4,22-stigmastadiene-3-one, and 3β-hydroxy-5-pregnen-20-one acetate.

Table II. Precision of Method

Run No.	Result, %
1	98.9
2	99.1
3	99.3
4	99.4
5	99.4
6	99.9
Average	99.3%
Standard deviation	±0.34%

95% confidence limits = ±0.34 × 2.571 = ±0.87%

An inspection of the curves of the rate studies (Figures 1 and 2) reveals that aldehydes are reduced almost instantaneously, while ketones are reduced at a much lower rate. Keto groups attached to or in the steroid molecule (Figure 3) are reduced even more slowly than the typical ketone, benzophenone (Figure 1). This significant difference in speed of reduction makes possible the determination of the aldehyde function in 3-ketobisnor-4-cholene-22-al. The rate curve of this compound (Figure 2) is the result of the total reduction of the aldehyde group and a partial reduction of the keto group. The ketone reduction curve is a straight line within the time of the experiment. The content of aldehyde function in 3-ketobisnor-4-cholene-22-al is then indicated on the ordinate axis by the point obtained when the straight-line portion of the rate curve between 5 and 60 minutes' reaction time is extended to intercept the zero time axis.

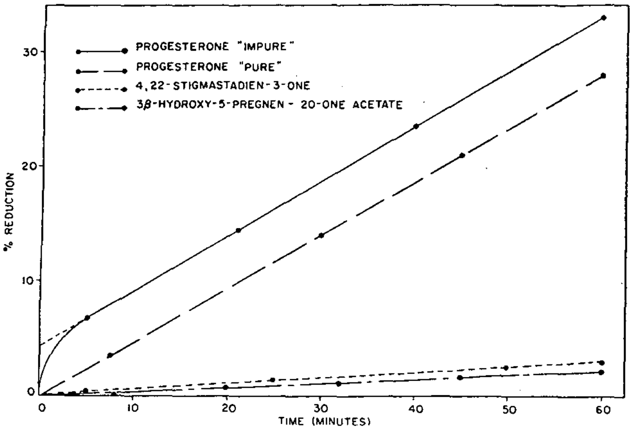


Figure 3. Rate of reduction of various keto steroids by sodium borohydride

This approach was used in the determination of 3-ketobisnor-4-cholene-22-al in known mixtures with 4,22-stigmastadien-3-one. The results of this experiment are shown in Table I. The data confirm the fundamental soundness of the procedure.

The precision of the method was studied by making six random determinations on the same lot of 3-ketobisnor-4-cholene-22-al. The results are shown in Table II.

The same approach was used in a determination of the purity of undried progesterone compared with dried material (Figure 3). An initial excessive consumption of sodium borohydride by the undried progesterone was found to be the result of an easily removable carbonyl-containing solvent.

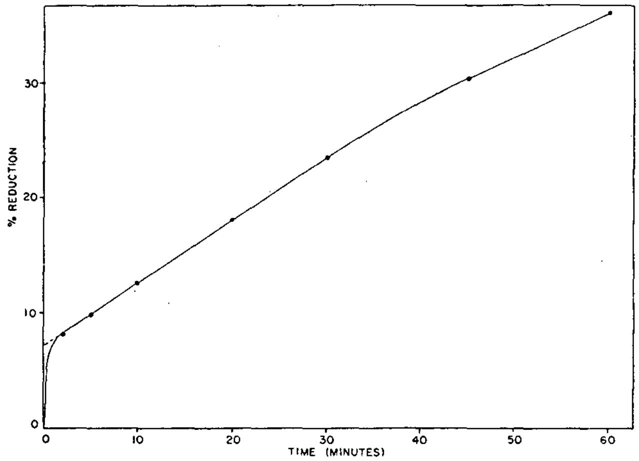


Figure 4. Rate of reduction of crude progesterone by sodium borohydride

This experiment suggested the use of a rate study of the reduction with sodium borohydride in order to detect and measure small amounts of 3-ketobisnor-4-cholene-22-al occurring in crude progesterone. Both paper strip chromatography and infrared spectroscopy identified this aldehyde as an impurity in crude progesterone. In order to determine if the 3-ketobisnor-4-cholene-22-al assay could be used for this purpose, a series of analyses were made on known mixtures of 3-ketobisnor-4-cholene-22-al in progesterone. The results are tabulated in Table III.

These results show that the method is accurate down to the 5% level of 3-ketobisnor-4-cholene-22-al in progesterone. For reasons that have not yet been explained, lower levels give high results.

Figure 4 shows the curve of a rate study on the reduction of a

Table III. Analysis of 3-Ketobisnor-4-cholene-22-al in Mixtures with Progesterone

3-Ketobisnor-4-cholene-22-al, %	
Added	Recovered
18.1	18.3
12.9	13.4
12.1	12.9
10.2	9.9
8.2	8.4
5.4	5.2
3.3	4.2
1.6	2.0

sample of crude progesterone with sodium borohydride. The sample contained 7.2% 3-ketobisnor-4-cholene-22-al.

ACKNOWLEDGMENT

The authors wish to thank J. L. Johnson for infrared measurements and interpretations and N. H. Knight for other valuable chemical measurements.

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Received for review June 1, 1954. Accepted November 11, 1954.

Determination of Sulfur in Volatile Hydrocarbon Mixtures by a Lamp-Conductometric Method

E. R. QUIRAM
Esso Laboratories, Research Division, Standard Oil Development Co., Linden, N. J.

A conductometric method has been developed for the determination of sulfur in the products of combustion resulting from lamp burning of volatile hydrocarbon mixtures. It is simpler than conventional procedures, and has eliminated many of the weaknesses encountered with turbidimetric, nephelometric, volumetric, and gravimetric methods. A new type of absorber has been designed which permits the determination to be completed in one vessel. Means are provided for eliminating errors caused by temperature fluctuations and dissolved carbon dioxide. The method has been found suitable for all ranges of sulfur concentration. The presence of aldehydes, halogens, nitrogen, and other acid-forming substances will interfere.

THE lamp method for the determination of sulfur in volatile hydrocarbon mixtures has been used for many years in the petroleum industry. The sample is allowed to burn from a wick-fed lamp and the products of combustion are absorbed in an aqueous solution of hydrogen peroxide which oxidizes the oxides of sulfur produced to sulfuric acid. This acid is then determined either volumetrically (*1*) or gravimetrically (*2*).

The volumetric procedure has been used to a greater extent than the gravimetric procedure, because it is simple and rapid. An objectionable feature of the volumetric method is that it is not satisfactory for measuring extremely low concentrations of sulfur. The turbidimetric procedure described by Zahn (*5*) permits the detection and estimation of as little as 0.0001% of sulfur; however, most refinery control laboratories are reluctant to use turbidimetric or nephelometric methods, because they are too time-consuming and require a considerable amount of skill and care on the part of the analyst.

Another feature of the volumetric procedure which has been a problem to refinery laboratories is the manner of predicting within reasonable tolerance the amount of sulfuric acid in the absorbing solution. If the material tested has a high sulfur content and a large sample is burned, the excessive amount of sulfuric acid formed will necessitate a long tedious titration. Brown (*3*) has overcome this difficulty by measuring the conductivity of the solution during burning. When a predetermined conductivity value is reached, the burning is discontinued. The conductivity reading by Brown's procedure indicates the approximate sulfur content of the sample, which then enables the operator to complete the analysis by conventional methods. Rae (*4*) investigated the possibility of determining sulfur in iso-octyl alcohol by means of a direct conductivity method, but the results were not entirely satisfactory. He believed that incomplete removal of sulfuric acid from within the fritted disk of the ASTM absorber was the cause of low results.

This paper describes a rapid conductivity method for the determination of sulfur in volatile hydrocarbon mixtures which eliminates many of the weaknesses encountered within turbidimetric, volumetric, and gravimetric procedures.

APPARATUS

Manifold System. The manifold system employed has been described in detail (*2*). A simplified technique for heating carbon dioxide is shown in Figure 1. This type of heating device is desirable when steam is not available.

Lamp Assembly. The lamp assembly is similar to that described in an ASTM method (*1*).

Cotton Wicking. Clean, uniform, unused cotton wicking in 7-inch lengths.

Conductance Bridge. Conductivity measurements were taken using the Serfass No. 3965 conductance bridge.

Conductivity Cell. A micro dipping type No. 3997 supplied by Arthur H. Thomas Co., Philadelphia, Pa. This type of cell can be inserted in a cylinder with a 0.5-inch inside diameter. The cell constant is approximately 1.0 reciprocal centimeter.

Temperature Bath. Any temperature bath is acceptable, as long as it will maintain a temperature of 25° ± 0.5° C.

Absorption Assembly, as shown in Figure 2.

REAGENTS AND MATERIALS

Carbon Dioxide and Oxygen. Each gas having a purity of not less than 99.5%, supplied in metal cylinders. Run a blank determination by bubbling the gases through the standard peroxide solution for a period of 1 hour and then determine the sulfur content by conductivity.

Hydrogen Peroxide, 3%. c.p. 30% hydrogen peroxide diluted with distilled water. Twenty milliliters of the diluted hydrogen peroxide solution should not contain over 0.015 mg. of sulfur.

Standard Sulfuric Acid Solution. Prepare 0.1249*N* sulfuric acid. This solution may be prepared as follows: Pipet 4.1 ml. of concentrated sulfuric acid into a 1-liter volumetric flask and dilute to the mark with distilled water. Standardize this solution against standard sodium hydroxide using methyl red indicator. This standard sulfuric acid solution should be approximately 0.15*N*. Remove exactly 500 ml. of the standard sulfuric acid and calculate the amount of distilled water which must be added to bring the final normality of the acid to 0.1249*N*. Add this calculated amount of water from a buret and mix thoroughly.

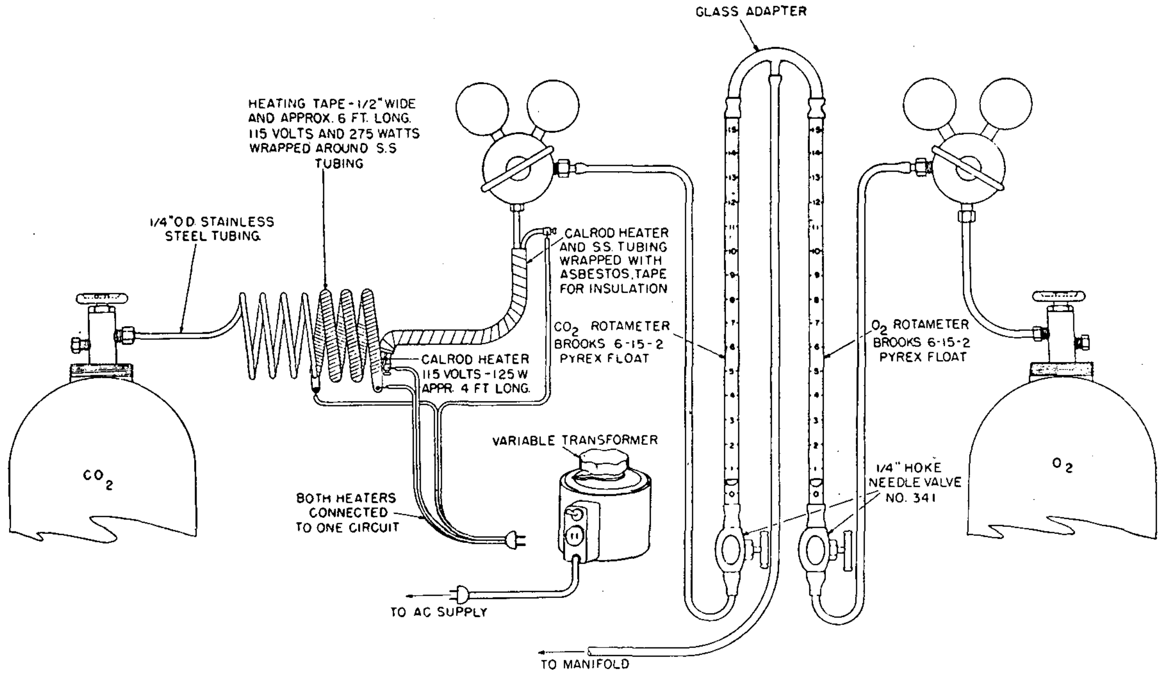

Figure 1. Details of heating and mixing apparatus

PROCEDURE

Preparation of Conductivity Standardization Curve. One milliliter of the standard 0.1249*N* sulfuric acid solution is equivalent to 2.0 mg. of sulfur. From aliquots of this solution, other standard solutions are prepared so that 1 ml. of each solution will contain 0.2, 0.02, and 0.005 mg. of sulfur, respectively. Suitable volumes of these solutions (0.005 to 12.0 mg. of sulfur) are added to 50-ml. glass-stoppered graduates and made up to a volume of 25 ml. with distilled water. If samples of extremely high sulfur content are anticipated, the standardization curve is extended beyond the 12.0-mg. limit. The graduates are stoppered and the contents are thoroughly shaken and the vessels are placed in a constant temperature bath, maintained at 25° ± 0.5° C. The conductivity cell is inserted in the graduate and the specific conductance of the solution is recorded. The concentration of sulfur is plotted against specific conductance of the solution on linear graph paper.

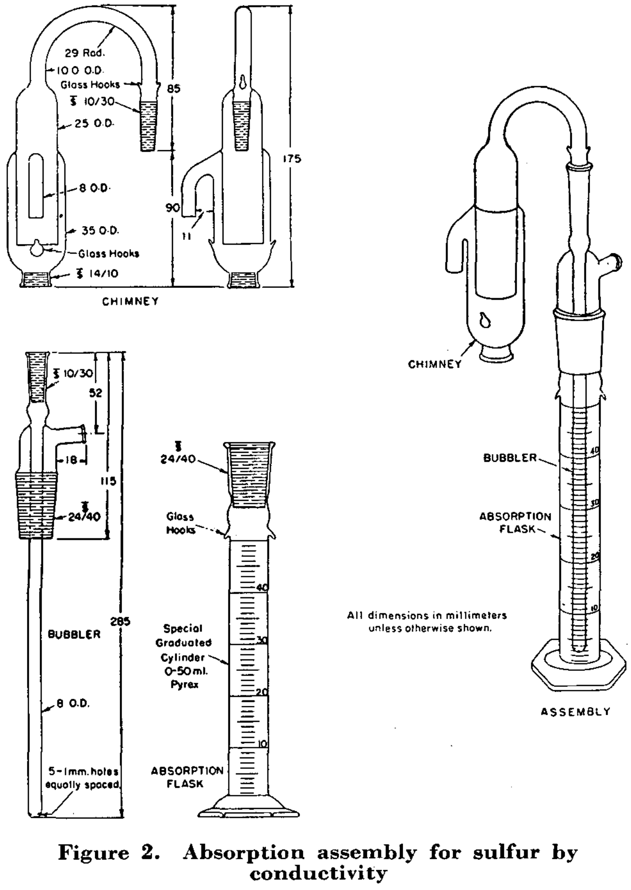

Figure 2. Absorption assembly for sulfur by conductivity

Burning Operation. Turn on the heater of the heating apparatus at least 10 minutes before using carbon dioxide.

Record the weight of a clean, dry, empty absorber and bubbler to the nearest 0.05 gram. Pipet approximately 20 ml. of freshly prepared 3% hydrogen peroxide into the absorber. The original absorber and bubbler weight can be recorded on the apparatus, so that subsequent weighings are not necessary.

Attach a chimney to the bubbler and connect it to the manifold by means of rubber tubing.

Prepare the burner by threading two strands of cotton wicking through the burner from bottom to top, cut them off evenly, and draw them down flush with the top of the burner.

Add from 5 to 10 grams of sample to the flask, insert the wick and burner, and allow the flask to stand until the wick has become primed with the sample. Weigh the assembled flask and burner to the nearest milligram.

Connect the side tube of the burner to the burner manifold by means of rubber tubing. Open the primary air needle valve to pass 1.0 to 2.0 cu. feet per hour of combustible gas through the burner. A combustion gas mixture of 65% carbon dioxide and 35% oxygen is suitable for most samples. Open the secondary needle valve to raise the total flow of gas to about 5 cu. feet per hour. Light the burner with a sulfur-free alcohol lamp, insert the burner into the chimney, and fasten it in place with a small spring.

Continue the combustion for approximately 1 hour. Cut off the combustion gas supply to the lamp, remove the burner and flask, and immediately reweigh. The difference in weight before and after the burning step represents the weight of the sample.

Lift the bubbler attachment slightly and add 4 to 5 ml. of distilled water to the absorbing solution in order to bring the total volume of solution to approximately 25 ml., which is the amount used in making the standardization curve. Weigh absorber plus bubbler and its contents to ±0.05 gram. The difference between this weight and the weight of the dry empty absorber plus bubbler indicates the weight of the solution in the absorber.

Place the absorber plus bubbler and its contents in a constant temperature bath maintained at 25° C. Bubble air through the absorbing solution for 5 minutes. This air should be scrubbed through $1N$ sodium hydroxide and bubbled in at a rate of 3 to 4 cu. feet per hour.

Insert the conductivity cell into the solution and record the conductivity reading.

Whenever a new peroxide solution is prepared or when a new cylinder of carbon dioxide or oxygen is used, run a blank determination.

CALCULATIONS

The sulfur content of the sample is obtained from the following equation:

$$\text{Per cent S} = (M_s - M_b) \times \frac{1}{10} \times \frac{W_a}{25} \times \frac{1}{W_s}$$

where

M_s = milligrams of sulfur corresponding to the conductivity reading of the sample
M_b = milligrams of sulfur corresponding to the conductivity reading of the blank
W_a = weight of absorbing solution in scrubber after burning
W_s = weight of sample in grams

RESULTS AND DISCUSSION

A desirable feature of the conductivity method is the simplicity and rapidity with which an analysis can be performed. When burning in a combustion atmosphere of 65% carbon dioxide and 35% oxygen approximately 3 to 5 grams of sample can be burned in 1 hour. The ratio of carbon dioxide to oxygen is varied, however, depending upon the components present in the fuel. An aliphatic type sample such as iso-octane will burn best when a ratio of 65% carbon dioxide and 35% oxygen is used, while an aromatic-type sample such as benzene burns best when the ratio is 77% carbon dioxide and 23% oxygen. The lower the oxygen content of the combustion mixture, the slower will be the burning rate. The results shown in Tables I and II were obtained under varying carbon dioxide to oxygen ratios, and indicate extremely good accuracy when compared with results obtained by conventional nephelometric and gravimetric procedures. Eight determinations can be completed in about 3 hours using eight apparatus assemblies, whereas conventional methods require a considerably longer time. Nephelometric and turbidimetric methods may take as long as 2 days.

Table I. Comparison of Conductivity and Nephelometric Lamp Sulfur Procedures

Sample No.	Per Cent Sulfur	
	Nephelometric	Conductivity
1	0.0002	0.0006
2	0.0002	0.0003
3	0.0007	0.0006
4	0.0025	0.0020
5	0.0039	0.0054 0.0051
6	0.0046	0.0055
7	0.011[a]	0.0104 0.0107 0.0106

[a] ASTM cooperative sample, tested by six cooperating laboratories by the proposed ASTM carbon dioxide-oxygen procedure (*2*). The average result of the laboratories was 0.011% of sulfur.

Table II. Comparison of Conductivity and Gravimetric Lamp Sulfur Procedures

Sample No.	Per Cent Sulfur	
	Gravimetric	Conductivity
1	0.024	0.023
2	0.024	0.024
3	0.026	0.028
4	0.027	0.029
5	0.036	0.037
6	0.057	0.057
7	0.060	0.068
8	0.066	0.068
9	0.073	0.074
10	0.075	0.077
11	0.084	0.084
12	0.089	0.088
13	0.092	0.087
14	0.097	0.104
15	0.097	0.092
16	0.100	0.096
17	0.479	0.472

Table III. Effect of Dissolved Carbon Dioxide on Conductivity of Peroxide Solution

Test No.	Condition of Test	Conductivity, Micromhos	Equivalent Sulfur Content, Mg.
1	Solution saturated with carbon dioxide	38	0.049
2	Solution saturated with carbon dioxide	37	0.047
3	Above saturated solution air blown	6.3	0.009
4	Above saturated solution air blown	8.0	0.012

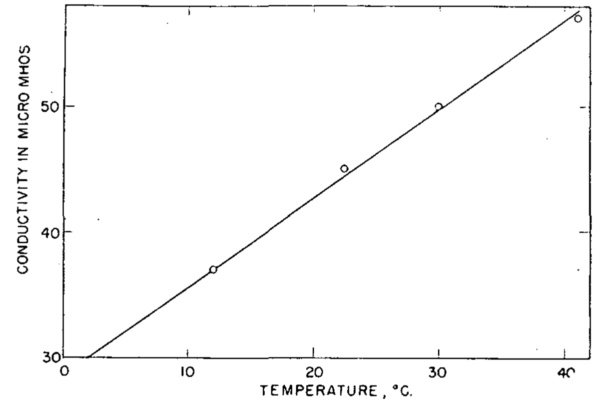

Figure 3. Effect of temperature on conductivity reading

Conductivity methods eliminate guesswork, as they do not require a previous knowledge of the amount of sulfuric acid formed. The method has been found eminently suitable for all ranges of sulfur concentration.

Absorber. Appreciable errors in conductivity measurements can be made with solutions having extremely small hydrogen ion concentrations. To minimize such errors it is desirable to work with a more concentrated acid solution. One means of accomplishing this end is by collecting the acid gases in an absorber containing a minimum amount of hydrogen peroxide solution. Selecting the proper type of absorber, however, presented a major problem. With the ASTM type absorber (*1*) a large volume of solution is required to remove all combustible gases efficiently. Evaporation of the absorbing solution to effect concentration was also considered, but was rejected because inherent errors are normally encountered when transferring solutions from one vessel to another. Figure 2 shows the type of absorber finally selected. The advantages of this type of absorber are: it is of simple design and can easily be made by a glassblower, the vessel is light in weight and readily adaptable for weighing on a balance, efficient recovery of combustible gases can be obtained when using a small volume of absorbing solution, means are provided for efficiently removing dissolved carbon dioxide, and conductivity electrodes fit conveniently into the absorption cylinder.

Rinsing the chimney is unnecessary. Experiments have shown that a quantity of sulfur too small to measure is retained in the chimney after combustion of nonleaded fuels.

Effect of Dissolved Carbon Dioxide. During the burning operation, the hydrogen peroxide solution becomes saturated with carbon dioxide, which is reflected in a marked increase in conductivity. Since any increase in conductivity resulting from dissolved carbon dioxide will be expressed in terms of sulfur, it is essential that excess carbon dioxide be removed. This is best accomplished by aerating the absorbing solution. Table III indicates the noticeable decrease in conductivity that can be obtained when a peroxide solution saturated with carbon dioxide is air blown for 5 minutes.

Effect of Temperature. Temperature is an important variable to be considered when making accurate conductivity measurements. Figure 3 indicates that an error of approximately 2.0% can be obtained with each 1° C. change in temperature. Obviously this is an appreciable source of error; therefore, all readings should be made in a carefully controlled temperature bath.

Interferences. The presence of halogens and nitrogen will interfere with this conductometric procedure. Purified air cannot be used in place of carbon dioxide–oxygen because oxides of nitrogen will form during the combustion of the sample. The most likely source of these interferences will be found in gasolines containing tetraethyllead (halogens present in scavenging agents), fuels from shale oil, and samples after hypochlorite treating. The presence of aldehydes, formed by the incomplete combustion of the gasoline, will also interfere and tend to increase conductivity. This may be caused by the formation of acids resulting from the oxidation of the aldehydes in a hydrogen peroxide solution. Ketones, on the other hand, are not easily oxidized and do not seem to interfere. The incomplete combustion of the gasoline may be ascertained by noting the appearance of the flame during the burning operation. If the flame tends to smoke or a change in the color of the absorbing solution is noted, the sample should be discarded.

ACKNOWLEDGMENT

The author wishes to acknowledge the able assistance of P. B. Gerhardt, G. H. Byrd, Jr., and E. R. Hartmann, who made many helpful suggestions and carried out much of the experimental work.

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RECEIVED for review June 8, 1954. Accepted November 12, 1954.

Volumetric Determination of Phosphorus

DAVID M. ZALL, EDWARD WAGMAN[1], and NATHAN INGBER[2]
U.S.N. Engineering Experiment Station, Annapolis, Md.

This work was initiated to find a simple and fast method for the determination of phosphorus in boiler compound. Boiler compound consists of a mixture of sodium carbonate, disodium phosphate, and starch. The specification limits this compound to these three constituents. Practically all of the procedures for the determination of phosphorus depend upon its precipitation by ammonium molybdate and the subsequent treatment of the yellow precipitate. The method described in this paper employs a simplified approach. The phosphate and carbonate are titrated in the presence of each other using the proper indicator. In cast iron, the phosphorus is first separated from the bulk of the iron with strong alkali. The filtrate containing the phosphate is neutralized and then titrated with standard alkali.

THE procedure described was developed during a search for a simple method of analysis for boiler compound. Boiler compound is used to treat boiler water and consists of a mixture of sodium carbonate, disodium phosphate, and starch. The methods employed for the determination of either one of the constituents when separated are well known and need no elaboration. However, in a mixture as found in boiler compound, the method for the determination of the constituents is more complicated. Similarly, the method for the determination of phosphorus in cast iron and phospho-organic compounds is subject to the same complications.

The present method for phosphate determination is long and tedious. It normally requires the initial separation of the phosphate as a phospho-molybdate precipitate. The phosphate in the precipitate may be determined either volumetrically or gravimetrically. In contrast, direct titration of phosphates by either acid or alkali provides accurate results which can be obtained very rapidly. The exact end points of these reactions and the reversible nature of the monosodium disodium phosphates are well known. For example, the pH of a solution of monosodium phosphate can be calculated from the K_1 and K_2 of phosphoric acid.

The proposed method employs a simple direct titration of phosphate by either acid or alkali. The results are accurate and rapid. The method is based on the reversibility of the monosodium-disodium phosphate equilibrium which has been discussed by Quimby (*7*). Helrich and Rieman (*6*) have determined phosphorus in phosphate rock by titration to a definite pH. Daĭn, Hal'perin, and Kolker (*2*) have determined disodium phosphate with the aid of a mixed indicator. Goudie and Rieman (*5*) used ion exchange to isolate the phosphoric acid from phosphate rock. Boos and Conn (*1*) used the method of Kolthoff and Cohn for the microdetermination of phosphorus.

The phosphate and carbonate in boiler compound are determined on the same sample. The carbonate is titrated with standard acid in the presence of the phosphate. The carbon dioxide formed in the carbonate titration must be eliminated to prevent interference in the subsequent phosphate titration. Dijksman (*3*) recommended elaborate precautions for elimination of carbon dioxide to prevent interference with the titration of phosphate. These precautions apply equally to the determination of phosphorus in cast iron and phospho-organic compounds.

RECOMMENDED PROCEDURE

Reagents. Standard sodium hydroxide (carbonate-free) and sulfuric acid solutions (0.1*N*). Phenolphthalein indicator (1% in alcohol) and methyl purple solution (obtainable from Fleisher Chemical Co., Benjamin Franklin Station, Washington 4, D. C.).

Procedure. Transfer a sample (aliquot) containing no more than 0.15 gram of sodium carbonate and no more than 0.15 gram of disodium phosphate to a 250-ml. Erlenmeyer flask. If the sample volume is less than 100 ml., dilute with distilled water

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[1] Present address, Navy Department, Bureau of Ships, Washington, D. C.

[2] Present address, Army Chemical Center, Edgewood, Md.

to a minimum of 100 ml. Add 4 drops of methyl purple indicator. Add from a buret standard sulfuric acid until the indicator changes from green to purple. Add 5 ml. more of standard sulfuric acid, and record the total volume of standard acid added. Place a few glass beads in the flask and boil vigorously on a hot plate, wash sides down with carbon dioxide-free distilled water, and cool in a water bath to room temperature. Titrate with standard sodium hydroxide to the first appearance of the green. Add 4 drops of phenolphthalein, and continue the titration to the appearance of a definite pink color which holds for at least 30 seconds.

$$(\text{Total ml. of } H_2SO_4 - \text{ml. of NaOH}) \times \text{normality} \times 0.053 = \text{grams of } Na_2CO_3$$

As an alternative, the carbon dioxide dissolved in the solution can be removed by vigorously bubbling carbon dioxide-free air or nitrogen through the solution for about 20 minutes. This would eliminate the necessity of boiling and subsequent cooling of the solution. After the carbon dioxide removal, the titration procedure is the same as indicated.

Determination of Phosphate. Determine the phosphate on the same sample. Back-titrate with standard sulfuric acid through the green color and to a definite purple color of the methyl purple indicator. Record the volume of sulfuric acid required for this titration. Calculate the phosphate content.

Phosphorus in Cast Iron. Transfer a 5-gram sample of cast iron into a 400-ml. beaker, cover with a watch glass, and dissolve with 50 ml. of dilute nitric acid (1 to 1). Add several drops of hydrofluoric acid and continue boiling to volatilize the silicon. After sample has dissolved, add 35 ml. of concentrated perchloric acid and heat until fumes appear. Continue to fume until salts of perchloric acid separate out and most of the acid has volatilized. Cool, rinse down watch glass and sides of beaker, and dilute with 75 ml. of water. Filter through No. 41 filter paper, and wash several times with hot water.

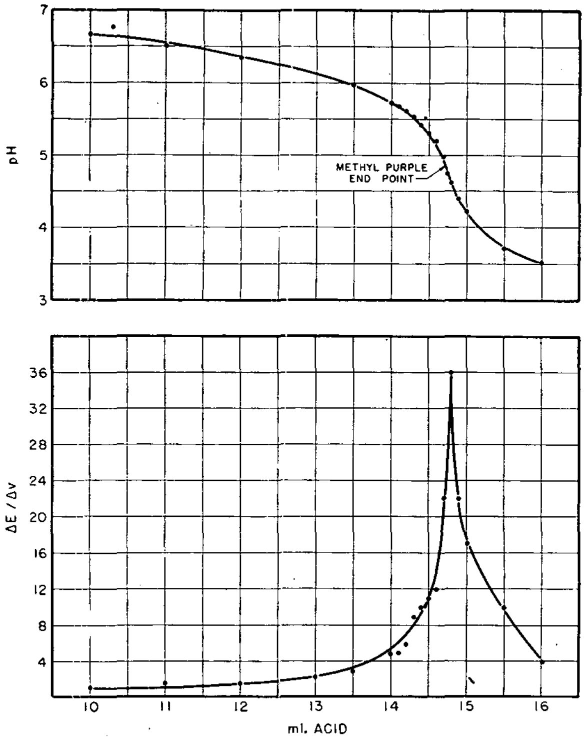

Figure 1. Titration of disodium phosphate with acid

Transfer filtrate to a separatory funnel. Add dropwise the solution from the separatory funnel into a 500-ml. volumetric flask containing 150 ml. of hot 50% sodium hydroxide solution. Rinse the separatory funnel twice with distilled water and add to main solution in volumetric flask. Allow to cool to room temerature, make up to mark, and mix well. Allow the precipitate to settle. Decant approximately 200 ml. of solution into a dry beaker. Filter decanted solution through a dry paper and funnel into a 200-ml. volumetric flask. Transfer exactly 200 ml. to a 400-ml. beaker.

Neutralize with dilute sulfuric acid (1 to 3) using phenolphthalein as indicator and add 5 ml. in excess. Boil to drive off carbon dioxide and continue boiling for about 15 minutes. Remove from hot plate and cool. Add 4 drops of phenolphthalein and neutralize carefully with dilute (1 to 5) sodium hydroxide, free of carbonate, until the appearance of a pink color. Add 4 drops of methyl purple, and titrate with standard sulfuric acid (0.05*N*) to the methyl purple end point. Calculate the amount of phosphorus in the sample.

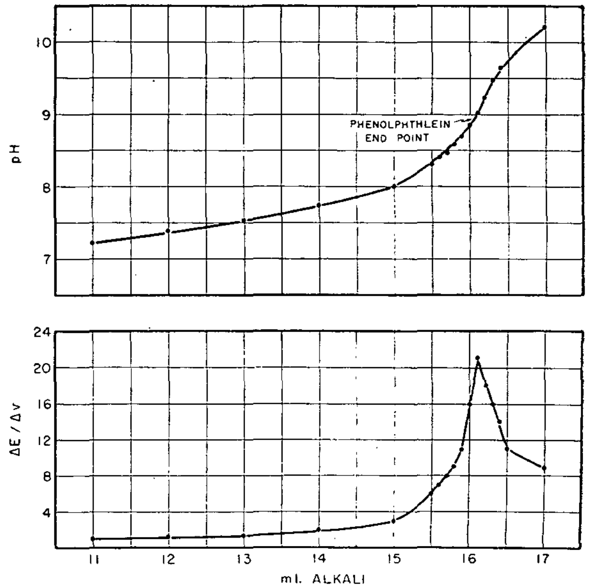

Figure 2. Titration of monosodium phosphate with alkali

As an alternative, for the separation of phosphorus from interfering elements the mercury cathode can be employed. The electrolysis can be performed in either sulfuric or perchloric acid medium. At the end of the electrolysis, drain off the electrolyte, wash the mercury with carbon dioxide-free water, and combine solution and washings. Bubble carbon dioxide-free air through the solution for about 15 minutes (to eliminate carbon dioxide). Add 4 drops of phenolphthalein and neutralize carefully with dilute (1 to 5) sodium hydroxide, free of carbonate, until the appearance of a pink color. Titrate the phosphate.

Phospho-Organic Compounds. Weigh a suitable sized sample (0.2 to 1.0 gram), depending on the phosphorus content, and place in the platinum cup of an oxygen bomb. Ignite the sample. Allow the bomb to cool off for about 10 minutes. Open the bomb, and transfer the ignited sample into a 400-ml. beaker, rinsing with carbon dioxide-free distilled water. Make alkaline with sodium hydroxide. Filter off any appreciable precipitate. Treat filtrate as described for cast iron and titrate with standard alkali.

DISCUSSION

In view of the reversible nature of the reaction disodium phosphate to monosodium phosphate, equivalent amounts of acid and alkali are required for the reactions.

$$Na_2HPO_4 + H^+ \leftrightharpoons NaH_2PO_4 + Na^+$$

$$NaH_2PO_4 + Na^+ \leftrightharpoons Na_2HPO_4 + H^+$$

These equations indicate that a double check can be made on the phosphate content, by titrating back with standard acid to the methyl purple end point.

In order to obtain additional information on the reversible reaction disodium-monosodium phosphate, the titrations were

Table I. Analysis of Synthetic Samples

Taken, Gram		Found, Gram	
Na_2CO_3	Na_2HPO_4	Na_2CO_3	Na_2HPO_4
0.1000	0.0000	0.0999	0.0000
0.1000	0.0250	0.1002	0.0255
0.1000	0.0500	0.1005	0.0507
0.1000	0.0750	0.1002	0.0750
0.1000	0.1000	0.1004	0.1002
0.1000	0.1250	0.1005	0.1245
0.0800	0.1250	0.0805	0.1246
0.0600	0.1250	0.0603	0.1251
0.0400	0.1250	0.0404	0.1250
0.0200	0.1250	0.0198	0.1250
0.0000	0.1250	0.0000	0.1248
0.0500	0.0625	0.0499	0.0623

studied electrometrically. The potentiometric titration curves, shown in Figures 1 and 2, indicate sharp breaks at both end points. In a somewhat similar titration Vermast (*8*) successfully titrated mono- and diammonium phosphate potentiometrically. Complete stoichiometric transformation of the reversible reaction was noted at the graphic inflection points.

With the possibility of the potentiometric titration of disodium phosphate definitely established, the effect of adding sodium carbonate was next investigated. As expected, the pH of the disodium phosphate–sodium carbonate mixture was considerably higher than that of the disodium phosphate alone. However, after titrating the carbonate and eliminating the carbon dioxide, the residual solution became essentially a mixture of phosphate and sulfate. The phosphate then was converted quantitatively to disodium phosphate by adding standard sodium hydroxide and noting the potentiometric end point. A double check was made by back-titrating with standard acid.

Because potentiometric titrations are not always practical for control work, suitable indicators were employed. A variety of indicators were studied, in order to establish sharp color changes corresponding to the inflection points on the curve. Phenolphthalein very closely approached the monosodium to disodium phosphate end point. The mixed indicator methyl purple (*4*) corresponded to the disodium to monosodium phosphate end point. Also, these two indicators were mutually compatible. The actual indicator end points as compared with the potentiometric end points are shown in Figures 1 and 2.

Initially even a simpler procedure was visualized. An attempt was made to titrate both the phosphate and carbonate in the presence of each other without eliminating the carbon dioxide. The solution of the two components was titrated with standard acid to the phenolphthalein end point. This titration represented the conversion of the carbonate to the bicarbonate. From the value thus obtained, the total carbonate content was calculated. The titration was then continued with standard acid to the methyl purple end point. The number of milliliters titrated between the phenolphthalein and the methyl purple end points represented the second half of the carbonate titration and the conversion of the disodium phosphate to monosodium phosphate. The phosphate content was thus calculated. However, even under the most favorable conditions of light and temperature, the phenolphthalein end point at the bicarbonate break was not sufficiently sharp and distinct. Many experimental runs were made, but the results obtained were not sufficiently reproducible. In routine analysis, the operators had difficulty with the color end point. This approach was therefore abandoned.

Then a dual titration was employed. Excess standard acid was added to the sample. The carbon dioxide was then driven off by boiling and the residual phosphate and excess acid were back-titrated with standard alkali. The carbonate was then determined by the difference in titration values.

This method applies only to a mixture of sodium carbonate and disodium phosphate. The presence of either sodium hydroxide or trisodium phosphate will interfere with the outlined procedure. However, their presence will be detected by the initial pH reading of the solution.

PRECISION AND ACCURACY

Synthetic mixtures of disodium phosphate and sodium carbonate were prepared and titrated by the developed method. In addition, samples of the pure materials were titrated to determine whether or not the method was applicable to pure materials. The results are shown in Table I.

Table II. Analysis of Boiler Compound Samples

Sample	Evolution Method Replicate samples		Volumetric Method Operator 1	Volumetric Method Operator 2
	Carbonate Content, %			
1	35.6	35.6	35.7	35.6
2	35.8	36.2	36.0	36.2
3	36.4	36.8	36.7	36.2
4	37.2	37.0	37.1	37.2
5	35.4	35.7	35.7	35.5
6	36.8	36.9	36.7	36.8
7	35.8	35.6	35.6	35.6
8	35.8	35.7	35.5	35.6
9	37.4	37.2	37.1	37.1
10	38.1	37.9	37.8	37.9
	Gravimetric Method Replicate samples			
	Phosphate Content, %			
1	48.6	48.9	48.7	48.5
2	46.9	46.8	46.5	46.3
3	46.8	46.6	46.5	46.5
4	45.4	45.5	45.2	45.3
5	48.8	48.9	48.7	48.6
6	46.4	45.9	45.7	45.8
7	47.6	47.8	47.4	47.4
8	46.4	46.6	46.3	46.3
9	45.6	45.4	45.5	45.4
10	46.4	46.4	46.3	46.2

Production samples of boiler compound were analyzed by the old methods (employed before this procedure was developed) and by this new method. The results are shown in Table II.

Standard samples of cast iron were analyzed by this method. Table III indicates the results obtained compared favorably with the certificate values of the samples.

Table III. Phosphorus in Cast Iron

N.B.S. Samples	Certificate Value, %	Volumetric Method, % No. 1	No. 2	No. 3
6a	0.431	0.429	0.430	0.432
7d	0.77	0.78	0.78	0.76
122a	0.307	0.310	0.303	0.305

ACKNOWLEDGMENT

The authors wish to express appreciation to R. C. Adams and F. E. Clarke for their helpful suggestions, and to M. Q. Garner for help in testing the procedure on inspection samples.

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Received for review June 7, 1954. Accepted November 13, 1954. Presented at the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., March 1954. The opinions expressed in this paper are those of the authors and do not necessarily reflect the views of the Navy Department or the Naval Service at large.

Ammonium Sulfamate as Substitute for Lead Peroxide in Microdetermination of Carbon and Hydrogen

A. S. HUSSEY, J. H. SORENSEN, and D. D. DeFORD

Department of Chemistry, Northwestern University, Evanston, Ill.

The oxides of nitrogen formed in the combustion analysis of nitrogen-containing compounds can be removed effectively by ammonium sulfamate dispersed on silica gel. This reagent, placed between the water absorption tube and the carbon dioxide absorption tube, removes oxides of nitrogen by reducing them to nitrogen and nitrous oxide. The use of this reagent eliminates the need for the lead peroxide section usually placed in the exit end of the combustion tube. The analytical values obtained when ammonium sulfamate is used are not notably more precise or more accurate than when a lead peroxide filling is used. However, the use of a substitute for lead peroxide eliminates the highly erratic values observed periodically when any of the equilibria involving the lead peroxide surface become upset by exposure of the filling to an excess of nitrogen oxides.

AMMONIUM sulfamate, dispersed on silica gel, has been used for several years in this microanalytical laboratory as a substitute for the lead peroxide which is usually employed to remove nitrogen dioxide from the combustion gas stream in microdeterminations of carbon and hydrogen (*7, 9*). This material functions as a reducing agent at room temperature according to the equation (*1, 5*):

$$2NO_2 + 2NH_4SO_3NH_2 \longrightarrow N_2 + N_2O + 2(NH_4)HSO_4 + H_2O$$

Because water is formed in this reduction, the reagent must be interposed between the water absorption tube and that for carbon dioxide, and provision must be made to prevent the transport of water into the carbon dioxide absorption tube.

A report recommending the use of trishydroxylammonium phosphate for this purpose has recently appeared (*3*). These investigators also considered the use of sulfamic acid but found it not to be of sufficiently high capacity, although its efficiency in removing nitrogen oxides was high.

The authors' greater success using ammonium sulfamate supported on silica rather than a mixture of asbestos, sulfuric acid, and sulfamic acid (*3*) is probably the result of elimination of channeling; the reaction product, ammonium bisulfate, is a solid salt and the granular support medium keeps the reagent uniformly dispersed during use. A modified Kraissel tube of borosilicate glass with a glass partition and interchangeable stopper, available as a stock item from glass apparatus suppliers, has been found particularly convenient for this absorption filling. The inlet side contains the ammonium sulfamate reagent; the exit side is filled with the same water-absorbing reagent that is used in the water absorption tube.

PREPARATION OF REAGENT

If 14- to 20-mesh silica gel is kept in contact with 2*M* ammonium sulfamate solution for several hours, then filtered and dried, it will have absorbed 1 to 3% of ammonium sulfamate. This material can then be used as a reagent to remove nitrogen dioxide. However, a material containing a higher percentage of ammonium sulfamate is preferable. This can be attained best by evaporating the bulk of the water used to disperse the reagent on the silica gel.

Sulfamic acid (97 grams, 1.0 mole) is dissolved with warming in 60 ml. of 28% ammonia (0.9 mole) which has been diluted with 50 to 60 ml. of water. This solution is added to 200 grams of 14- to 20-mesh silica in a heavy-walled filter flask and the two materials are mixed well. About two thirds of the water is removed at room temperature on the water pump. The mixture is then filtered, air dried, and finally dried at 105° to 110° C. for 8 hours. When so treated the silica retains 15 to 25% of its weight of ammonium sulfamate.

This reagent is placed in the inlet side of a Kraissel tube and the same reagent which is used in the water absorption tube is placed in the outlet side. Glass wool plugs are inserted at the side arms and top.

When stored between periods of use, the side containing the water-absorbing agent is kept capped; the ammonium sulfamate side is open to the air. A completely dry sulfamate reagent appears to be slightly less efficient than one which retains a slight amount of moisture.

The reagent remains effective for a 100 or more analyses. However, the authors replace the filling whenever the absorption tubes are changed in order to keep the water-absorbing agent in the exit side of the Kraissel tube exactly the same as that in the water absorption tube. By doing so, it is then possible to omit the sulfamate tube without upsetting the balance of the absorption tubes when samples known not to contain nitrogen are being analyzed.

APPARATUS AND PROCEDURE

The combustion train in use is composed of standard parts (*7, 9*)—medicinal grade oxygen passes through Tygon tubing to preheater, thence through a modified Kraissel tube containing Ascarite and Anhydrone into a standard microcombustion tube. Samples weighing 2 to 5 mg. are burned at 700° to 900° C. for 12 minutes, followed by 8-minute sweep out, using a semiautomatic combustion apparatus. A copper oxide–vanadium pentoxide combustion filling, as refined by Shelberg (*6, 8*), has been particularly successful and was used in these analyses. When lead peroxide cubes, prepared according to Shelberg (*8*) are used, the oxygen flow is regulated to a total volume of 240 ± 10 ml.; when ammonium sulfamate is used, the flow is increased to a total volume of 300 ± 10 ml. This is necessary in order to sweep all of the carbon dioxide through the longer path presented by the

Table I. Repeat Analyses of Analytically Pure Compounds

	Calculated, %		Found, %			
			Ammonium Sulfamate		Lead Peroxide	
Compound	H	C	H	C	H	C
2-(*p*-toylsulfonyl)-cyclohexanone 2,4-dinitrophenylhydrazone	4.66	52.77	4.7	52.4	4.6	52.7
			4.7	52.6	4.6	52.7
			4.8	52.4	4.7	53.2
			4.5	52.6	4.8	52.7
Av. error			−0.02	−0.30	−0.02	+0.02
Variance			0.016	0.013	0.010	0.053
Ammonium 4′-dimethylamino-4-azobenzene-sulfonate	5.64	52.16	5.5	52.3	5.4	52.3
			5.7	52.1	5.6	52.5
			6.1	52.2	5.7	52.4
			5.8	52.0	5.5	52.0
			5.6	51.9	5.7	52.3
Av. error			+0.04	0.00	−0.15	+0.20
Variance			0.047	0.025	0.017	0.035

Table II. Analysis of Diverse Research Compounds

Compound	Found, %		Theory, %	
	H	C	H	C
Lead Dioxide Method				
o-(Chloromethylsulfonyl)benzopiperidide	5.4	52.2	5.4	51.7
S-(2-Hydroxypropyl)isothiuronium *p*-tolylsulfonate	5.7	43.0	5.6	43.3
2-Methoxy-4-hydroxymethyl-6-methylnicotinic lactone	5.1	60.4	5.0	60.4
1-Aceto-2-phenylsulfonyl-1-phenylhydrazine	5.0	57.9	4.9	57.9
1,6-Dimethyl-4-hydroxymethylnicotinic lactone	4.7	60.4	5.0	60.3
N-(1-Ethyl-2-hydroxybutylidene)alanine	9.3	58.2	9.1	57.8
m-Nitro-α-acetophenonetripropionic acid	5.0	53.8	5.0	53.5
N-1-Ethyl-2-nitropropylideneaniline	7.5	64.6	6.8	64.1
N-1-Ethyl-2-nitroethylideneaniline	6.4	62.9	6.3	62.5
N-1-Methyl-2-nitropropylideneaniline	6.3	62.6	6.3	62.5
2-Nitro-3-(1-piperidino)benzothiophene	5.5	60.2	5.4	59.5
S-(*p*-Chlorobenzyl)isothiuronium 2-methyl-2-cyclopentene-1-sulfonate	5.4	46.6	5.3	46.3
S-(2-Sulfo-1-phenylethyl)isothiourea	5.0	41.9	4.7	41.5
S-Benzylisothiuronium α-tolylsulfonate	5.3	53.4	5.4	53.2
Phenylammonium 2-methyl-2-cyclopentene-1-sulfonate	6.8	56.6	6.7	56.4
Av. error			+0.10	+0.25
Variance			0.044	0.061
Ammonium Sulfamate Method				
Ethyl *p*-bromophenylhydrazonoacetylglyoxylate	4.1	46.0	4.2	46.0
S-(2-Hydroxycyclohexyl)isothiuronium sulfate	6.5	38.0	6.7	37.7
S-(2-Hydroxycyclopentyl)isothiuronium sulfate	6.8	34.4	6.3	34.4
m-Methylsulfonylphenyl 3,5-dinitrobenzoate	2.8	45.7	2.8	45.9
Methyl *p*-methoxyphenyl sulfone	4.9	51.8	5.4	51.6
N-Ethyl-5-hydroxydecahydroisoquinoline picrate	5.6	49.5	5.8	49.6
N-Ethyl-5-ketodecahydroisoquinoline picrate	5.4	49.7	5.4	49.8
p-Methylmercaptophenyl 3,5-dinitrobenzoate	3.1	50.4	3.0	50.0
4,5-Diphenyl-4-benzylpentyl 3,5-dinitrobenzoate	5.4	71.1	5.4	71.0
2-(*p*-Hydroxyphenyl)pyrrocoline	5.3	80.1	5.3	80.4
2-Phenyloctahydropyrrocoline hydrobromide	7.1	59.4	7.1	59.6
5-Methoxy-1,9-tetramethylene-1,2,3,4-hydrocarbazolenine picrate	4.9	56.9	5.0	57.0
S-(*p*-Chlorobenzyl)thiuronium 1-methyl-1-cyclohexene-6-sulfonate	5.7	47.8	5.6	47.8
α-Pinene isothiuronium *p*-tolylsulfonate	7.1	56.5	7.3	56.3
β-Pinene isothiuronium *p*-tolylsulfonate	7.2	56.3	7.3	56.3
Av. error			0.00	−0.03
Variance			0.044	0.036

Kraissel tube. When the lead peroxide section is omitted from the tube filling, the authors fill this section with a silver plug, either gauze or silver wool, maintained at 175° C. by means of a heating mortar.

Only one other modification is required when ammonium sulfamate is employed—the use of a heater for the inlet capillary tip of the water tube. This prevents the retention of some of the nitrogen dioxide in the liquid phase water which tends to condense here if the heater is not used (*2*, *4*, *6*).

All hydrogen values are obtained from the weight of water formed less 0.100 mg. The necessity for this standard correction is apparently derived from the condition of humidity in this laboratory and its validity has been demonstrated over several thousand analyses. The correction is constant for the lead peroxide method, for the ammonium sulfamate method, and in the absence of these reagents with nonnitrogenous samples.

RESULTS

The carbon and hydrogen values obtained from repeated analyses of two very carefully purified compounds permit a comparison of the relative effectiveness of ammonium sulfamate and lead peroxide in the removal of nitrogen dioxide (Table I). Variances of these results have been calculated from the formula (*10*)

$$\text{Variance} = \Sigma \frac{[(x - T) - D]^2}{n - 1}$$

where x is the value of individual measurement, T is the true (theoretical) value, D is the average error by each method, and n is the number of measurements.

In Table II carbon and hydrogen values are obtained from the analyses of 30 research compounds submitted to the laboratory. Fifteen of these, chosen at random, were analyzed by the authors' standard procedure using lead peroxide; fifteen, using ammonium sulfamate on silica. The variance for each method has been calculated as above, using as the average error, D, the average of the differences between the observed and calculated values. The assumption of purity of all the samples is implicit in the variances so calculated. Since a relatively large number of samples are involved, an individual sample of less than analytical purity would not greatly affect the validity of these variances as measures of precision of the methods.

The accuracy and the precision of the two methods (Tables I and II) are not significantly different. A slight advantage appears to reside in the method employing ammonium sulfamate rather than lead peroxide. However, the use of ammonium sulfamate has been found to eliminate almost entirely the extremely erratic carbon–hydrogen values which occur periodically when a lead peroxide filling is used in the combustion tube. This improvement is most obvious when many of the compounds being analyzed contain nitrogen or when a compound has an unusually high nitrogen content. This is the principal advantage of the method.

Pooled variances (*10*) of the analytical values in Tables I and II are hydrogen 0.034 and carbon 0.026. The corresponding pooled variances of the analytical values for cyclonite, picric acid, and 2,4-dinitrophenylhydrazine [Table III, (*3*)] are hydrogen 0.003 and carbon 0.018. The accuracies of the analytical determinations using ammonium sulfamate or trishydroxylammonium phosphate (*3*) are not significantly different for either carbon or hydrogen. The precision of hydrogen determinations is better by the Cross and Wright method but it is unlikely that this is a result of the nitrogen oxide absorbing reagent used. The precision of carbon determinations is of the same order by both methods.

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RECEIVED for review August 20, 1954. Accepted November 5, 1954. Abstracted in part from the thesis presented by Joyce H. Sorensen to the Graduate School of Northwestern University in partial fulfillment of the requirements for the degree of master of science, August 1952.

Determination of Microgram Quantities of Strontium in Solution

Evaluation of Flame Spectrophotometric Method

ALBERT E. TAYLOR and HAROLD H. PAIGE
Idaho State College, Pocatello, Idaho

Because of the widespread occurrence of small quantities of strontium, its chemical resemblance to calcium, and its occurrence in the bones of man and animals, there is a need for reliable methods to detect and measure microgram quantities of this element. A study of flame photometric methods including two different fuels, acetylene and hydrogen, and different light-measuring devices has been carried out. A few interfering elements have been included in the study. A statistical evaluation of the results indicates the general superiority of a photomultiplier detection unit (Photovolt Model 520-M), acetylene as the fuel, and a wave length of 681 mμ with a Beckman Model DU flame photometer attachment. Average standard deviations of 0.5 p.p.m. or less in the range 0 to 10 p.p.m. strontium have been obtained. The method can be readily applied to solutions containing strontium in this range after preliminary experience with the equipment and interferences.

STRONTIUM occurs widely to the extent of about 0.02% in the earth's crust, and on the average there appears to be about 0.5% as much strontium as calcium in agricultural soil. Because of its chemical resemblance to calcium, and hence because of its availability to plants in appreciable quantities and its occurrence in the bones of man and animals, there is need for reliable methods to detect and measure microgram quantities of this element.

A survey of available literature failed to disclose any thorough investigation for a rapid determination of strontium in solution at concentrations of 0 to 10 p.p.m. The flame photometric method would seem to be most promising for this purpose. Its use is mentioned by Beckman Instruments, Inc. (*1*), and Hinsvark and associates (*3*). In the latter case the minimum quantity of strontium measured was over 200 p.p.m. It was decided to investigate the following factors which may affect the determination by this method: wave length, type of detection unit, type of fuel and its pressure, and interferences by a few common elements.

EXPERIMENTAL

Method. The interferences of five different ions—calcium, sodium, magnesium, aluminum, and ferric—on the determination of strontium in the range of 0 to 10 p.p.m. strontium were studied at three fixed ratios to the quantity of strontium taken. Of these three ratios, the first was chosen after consulting United States Geological Survey publications (*4*) to obtain the maximum ratios of these ions which occur in natural waters throughout the United States. This maximum was substantially increased, ranging from approximately threefold for sodium ion to 66-fold for ferric ion. The other two ratios were chosen for purposes of determining interferences: one represented an arithmetic ratio—e.g., 0 to 10 p.p.m. strontium plus 1000 p.p.m. test ion; the other represented equal parts per million of each, strontium and test ion. No solutions were examined with combinations of these interfering elements. The solutions were examined with a Beckman DU quartz flame spectrophotometer, utilizing as detection units the phototubes and a Model 520-M Photovolt photomultiplier photometer. Each of the fifteen solutions, as well as the strontium alone, was atomized into both hydrogen and acetylene flames and intensities were measured at each of two wave lengths, 461 and 681 mμ. A graph was plotted for each set and the average standard deviation of strontium was determined for each throughout the range of 0 to 10 p.p.m. strontium as determined from the straight line most nearly connecting the experimentally derived points.

Table I. Determination of Strontium Using Photomultiplier Unit

	Strontium Taken, Parts per Million											
	10		7		5		3		1		0	
Conditions[a]	Sr found, p.p.m.	Std. dev.	Sr found, p.p.m.	Std. dev.	Sr found, p.p.m.	Std. dev.	Sr found, p.p.m.	Std. dev.	Sr found, p.p.m.	Std. dev.	Sr found, p.p.m.	Std dev.
Sr only												
0.6-mm. slit	9.85	0.15	7.00	0.13	5.05	0.13	3.15	0.15	0.90	0.16	−0.05	0.13
Sr + Ca												
1000 p.p.m., 461 mμ, 0.06-mm. slit	10.20	0.60	6.95	0.33	4.90	0.13	3.20	0.31	1.00	0.39	0.05	0.16
400 × p.p.m. Sr, 461 mμ, 0.05-mm. slit	9.93	0.19	7.20	0.15	5.07	0.15	3.36	0.27	1.10	0.04	−0.08	0.21
1 × p.p.m. Sr., 0.6-mm. slit	9.90	0.12	6.92	0.07	5.17	0.07	3.15	0.09	1.10	0.11	0.00	0.13
Sr + Na												
1000 p.p.m., 0.5-mm. slit	10.15	0.32	6.80	0.32	4.90	0.28	3.05	0.42	1.15	0.53	−0.30	0.63
400 × p.p.m. Sr, 1.0-mm. slit, 12 lb. O_2, 5 lb. H_2	9.65	0.11	7.00	0.13	5.10	0.07	3.16	0.12	0.55	0.06	−1.75	0.09
1 × p.p.m. Sr 0.5-mm. slit	9.63	0.19	7.33	0.17	5.50	0.17	3.18	0.19	1.50	0.21	−0.20	0.28
Sr + Mg												
200 p.p.m., 2.0-mm. slit, 12 lb. O_2, 6 lb. H_2	10.30	0.28	6.95	0.16	4.85	0.18	3.00	0.28	1.40	0.15	0.45	0.17
100 × p.p.m. Sr, 0.5 mm. slit	9.90	0.38	7.45	0.18	5.20	0.21	3.00	0.15	1.15	0.00	−0.10	0.22
1 × p.p.m. Sr, 0.7-mm. slit, 10 lb. O_2, 4 lb. C_2H_2	9.95	0.21	7.18	0.19	5.30	0.15	3.23	0.25	1.15	0.16	−0.13	0.34
Sr + Al												
100 p.p.m., 2.0-mm. slit, 12 lb. O_2, 7 lb. H_2	10.95	0.44	6.20	0.49	4.13	0.26	2.70	0.27	1.20	0.45	−0.20	0.29
50 × p.p.m. Sr, 2.0-mm. slit, 12 lb. O_2, 7 lb. H_2	10.35	0.33	6.80	0.20	4.80	0.27	2.80	0.20	0.80	0.16	0.30	0.55
1 × p.p.m. Sr, 0.8-mm. slit, 10 lb. O_2, 4 lb. C_2H_2	10.35	0.49	6.80	0.36	5.00	0.00	3.15	0.00	1.70	0.36	−0.40	0.43
Sr + Fe												
100 p.p.m. 0.7-mm. slit, 10 lb. O_2	10.25	0.15	6.80	0.25	5.15	0.26	3.20	0.27	1.35	0.36	−0.20	0.51
50 × p.p.m. Sr, 0.5-mm. slit, 10 lb. O_2, 4 lb. C_2H_2	9.85	0.08	7.05	0.07	5.13	0.09	3.27	0.05	0.87	0.06	−0.05	0.00
1 × p.p.m. Sr, 0.6-mm. slit, 10 lb. O_2, 4 lb. C_2H_2	9.65	0.24	7.25	0.21	5.50	0.13	3.30	0.30	1.10	0.35	−1.00	0.34

[a] Conditions. 681 mμ, 8 lb. O_2, 4 lb. C_2H_2 except as indicated.

Table II. Effect of Interfering Elements

Interfering Element[a]	Concn. of Other Element	Photo Detection Unit	Wave Length, Mμ	Slit Width, mm.	Fuel	Av. Std. Dev.
None	0	Photomultiplier	461	0.06	C_2H_2	0.37
			681	2.00	H_2	0.28
Calcium	1000 p.p.m.	Photomultiplier	681	0.40	C_2H_2	0.39
	400 × p.p.m. Sr	Photomultiplier	681	0.90	H_2	0.08
			681	0.30	C_2H_2	0.10
		Red phototube	681	0.30	C_2H_2	0.36
	1 × p.p.m. Sr	Photomultiplier	461	0.06	C_2H_2	0.19
			681	1.80	H_2	0.22
		Red phototube	681	0.30	H_2	0.39
Sodium	1000 p.p.m.	Photomultiplier	461	0.06	C_2H_2	0.38
		Red phototube	681	0.40	C_2H_2	0.32
	400 × p.p.m. Sr	Photomultiplier	461	0.30	H_2	0.08
			461	0.06	C_2H_2	0.34
			681	0.40	C_2H_2	0.14
		Blue phototube	461	0.30	H_2	0.30
			461	0.30	C_2H_2	0.35
	1 × p.p.m. Sr	Photomultiplier	461	0.06	C_2H_2	0.23
			681	2.00	H_2	0.33
Magnesium	200 p.p.m.	Photomultiplier	461	0.06	C_2H_2	0.49
			681	0.50	C_2H_2	0.30
	100 × p.p.m. Sr	Photomultiplier	461	0.70	H_2	0.45
			461	0.06	C_2H_2	0.28
			681	2.00	H_2	0.17
	1 × p.p.m. Sr	Photomultiplier	461	0.07	C_2H_2	0.40
			681	1.80	H_2	0.22
Iron	100 p.p.m.	Photomultiplier	461	0.07	C_2H_2	0.30
		Red phototube	681	0.30	C_2H_2	0.48
	50 × p.p.m. Sr	Photomultiplier	461	0.50	H_2	0.10
			461	0.06	C_2H_2	0.45
			681	1.50	H_2	0.06
		Blue phototube	461	0.30	H_2	0.35
		Red phototube	681	0.30	H_2	0.43
			681	0.30	C_2H_2	0.30
	1 × p.p.m. .Sr	Photomultiplier	461	0.80	H_2	0.43
			681	2.00	H_2	0.37
		Red phototube	681	0.30	C_2H_2	0.20

[a] Strontium taken, 0 to 10 p.p.m.

Apparatus. A Beckman DU quartz spectrophotometer with a Model 9200 Beckman flame attachment was used, both with and without a photomultiplier detection unit. The photomultiplier attachment was a Model 520-M Photovolt unit, replacing the phototubes of the Beckman instrument in a manner similar to that described by Collier and Barschel (*2*).

Reagents. Materials used were of reagent grade. Anhydrous sodium carbonate, calcium carbonate, magnesium carbonate, strontium carbonate, aluminum metal foil, and hydrogen-reduced iron powder were used in the preparation of the stock standards. Exchange resin–demineralized water was used.

Procedure. A stock standard containing 1000 p.p.m. of strontium was prepared by dissolving 1.6848 grams of strontium carbonate in 3*N* hydrochloric acid and diluting with 3*N* hydrochloric acid to 1 liter in a volumetric flask. Stock standards of 5000 p.p.m. were prepared by dissolving in 3*N* hydrochloric acid 2.3044 grams of sodium carbonate, 2.5000 grams of calcium carbonate, 3.4674 grams of magnesium carbonate, 1.0000 gram of pure iron powder, and 1.0000 gram of aluminum metal foil, respectively, and diluting with 3*N* hydrochloric acid to 200 ml. in volumetric flasks The strontium solution, which was used in the preparation of the final samples for testing, contained 50 p.p.m. of strontium and was prepared by a 1 to 20 dilution of the original stock with 3*N* hydrochloric acid. The 5000–p.p.m. stock standards of the test ions were used directly in preparing the final samples for testing, except in those samples containing equal parts per million of strontium and test ion. In preparing the latter, the original 5000–p.p.m. stock solutions were cut to 50 p.p.m. by a 1 to 100 dilution with 3*N* hydrochloric acid.

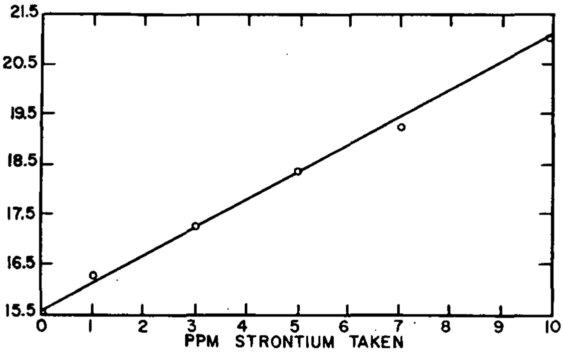

Figure 1. Strontium plus 100 p.p.m. iron

681 mμ, photomultiplier
0.7 slit, 10 pounds oxygen, 4 pounds acetylene

The final samples for testing were prepared by placing in 50-ml. volumetric flasks, respectively, 10, 7, 5, 3, 1, and 0 ml. of 50 p.p.m. strontium, adding the necessary amount of test ion to give the desired ratio, and then diluting to 50 ml. with water.

These samples were atomized through the flame with the photomultiplier unit at maximum sensitivity (switch in position *x*1). The intensity of the flame from each sample burned with hydrogen and with acetylene was measured at 461 and 681 mμ. A series of ten intensity readings was taken from each sample. The ten readings were averaged and a graph was made showing average intensity reading versus parts per million of strontium taken (Figure 1 is representative). The apparent parts per million of strontium found can now be read directly from observed flame intensities. Part of these data appears in Tables I and II.

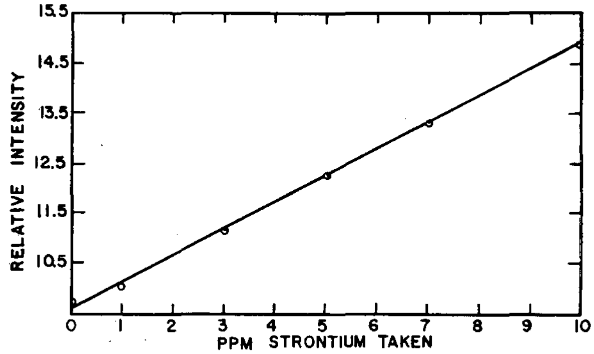

Figure 2. Strontium plus sodium (400 × p.p.m. strontium)

461 mμ, blue phototube
0.3 slit; 10 pounds oxygen, 4 pounds acetylene

This procedure was repeated using first the blue-sensitive phototube at 461 mμ, then the red-sensitive phototube at 681 mμ as a detection unit. In these determinations the selector switch was placed in position 0.1, while the sensitivity control was maintained at five turns from the extreme clockwise position for the acetylene flame and in the maximum counterclockwise position for the hydrogen flame. Six readings were taken for each sample and were treated as described in the preceding paragraph (Figure 2 is representative).

RESULTS

Table I shows the results in the determination of strontium in solution by flame photometry over the range of 0 to 10 p.p.m. This table shows the best combination of conditions (wave length used, type of fuel and its pressure, slit width, and type of detecting unit) for the determination of strontium in the presence of some common interfering elements. In each series the average error is less than 0.55 p.p.m., and the average standard deviation is less than 0.5 p.p.m.

Table II includes a statement of operating conditions not included in Table I which gave results having average standard deviation not greater than 0.5 p.p.m. in the range 0 to 10 p.p.m. strontium by flame photometry. The maximum average error in this group is 0.55 p.p.m.

DISCUSSION

For the flame photometric determination of strontium, the superiority of the Photovolt photomultiplier photometer over blue- and red-sensitive phototubes has been demonstrated in the range of 0 to 10 p.p.m. for the examples studied. In addition to the data given in detail in Table I for 16 series, there were 26 others utilizing this method which satisfied the criteria for inclusion in Table I, while only ten phototube series of the 64 studied gave results within these limits. In addition, only eight series with the photomultiplier as detection unit gave average standard deviations greater than 1 p.p.m., whereas 43 of the series studied with the phototube unit were beyond this value.

Acetylene was superior as a fuel in most cases where the photomultiplier detection unit was employed.

The wave length at which the most reliable results were obtained appeared to be 681 mμ, with the exception of those involving high concentrations of calcium.

No study was undertaken of the effect of mixtures of interfering elements nor of the use of other types of detecting units such as the Beckman photomultiplier attachment.

Figures 1 and 2 representing the relationship of relative intensity to strontium concentration indicate the manner in which readings on the two detection units used are translated into parts per million of strontium.

Repetition of the procedure in selected cases, including the preparation of new solutions and standards, gave results agreeing with those tabulated within the stated limits.

It appears unlikely that the flame photometric determination of strontium by these methods can be carried to a lower range of concentration than that included in this study.

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RECEIVED for review March 6, 1954. Accepted November 8, 1954. Based on work done for the Atomic Energy Commission under contract AT(10-1)-310 with Idaho State College.

Microdetermination of Cobalt in Biological Materials

BERNARD E. SALTZMAN
Division of Special Health Services, U. S. Department of Health, Education and Welfare, Cincinnati, Ohio

The spectrophotometric determination of low levels of cobalt in biological materials of high ash content has previously been hampered by difficulties with interfering metals and by losses of cobalt because of the occurrence of a precipitate at the pH required for complex formation. A separation procedure has been developed in which cobalt is complexed with 1-nitroso-2-naphthol under conditions which prevent the formation of a precipitate, and then is extracted with chloroform. The extract is purified by washing with dilute hydrochloric acid, and ashed to yield cobalt practically free from any interfering metal. Final determination is made by a nitroso R salt method which has been improved to give reliable results and very close adherence to Beer's law. Recovery of microgram quantities of cobalt added to 25-gram samples of bone was 95%. The effect of interfering metals has been reduced to a negligible level. The procedure makes possible accurate analysis of materials of high ash content.

DURING the course of a toxicologic study, the need arose for the determination of the normal levels of cobalt in tissue and bone. Since these were extremely low (later found to be of the order of 0.01 to 0.1 p.p.m.), a method was required with the highest possible sensitivity, which would tolerate large quantities of extraneous salts and which would be as free as possible from error due to interfering metals. These requirements were not met (especially for bone samples) by the commonly used nitroso R salt method (*4, 6, 7–10, 13, 17*), in which the reagent is applied directly to a solution of the ashed sample to develop a color with cobalt. Sensitivity was inadequate because the physical limits of the solubility of the ashed salts prevented the concentration of the color to the level required. Attempts to concentrate the cobalt–nitroso R salt complex by extraction with various solvents were unsuccessful. The extraneous salts, such as calcium phosphate in bone samples, caused incomplete recovery of cobalt because of the formation of heavy precipitate, to the point of solidification, at the pH required for color development. Finally, the specificity of the method was not adequate for the very low level of cobalt to be determined. Similar difficulties were encountered with spectrographic and other colorimetric methods (*1–3, 11, 19*). An investigation was therefore undertaken to develop a method of analysis which would meet the severe requirements imposed by bone samples.

It was evident that most of these problems would be solved if the cobalt could be separated from the ashed salts. Separation by precipitation in acid media with 1-nitroso-2-naphthol (*16, 18*) was not regarded as suitable for microgram quantities of cobalt because of the well-known problems of supersaturation. Separation by extraction using dithizone (*11*), 2-nitroso-1-naphthol (*2*), *o*-nitrosocresol (*3*), or 1-nitroso-2-naphthol (*5, 12, 14*), was more suitable for small quantities of cobalt, but only the latter reagent could be used at a sufficiently low pH to avoid precipitation of calcium phosphate with bone samples. Extraction with 1-nitroso-2-naphthol was therefore considered as a means for separating small amounts of cobalt from high-ash materials.

Little work has been reported with this reagent, although the bulk of literature on the microdetermination of cobalt is considerable. Paulais (*14*) removed impurities from the cobalt extract by washing with dilute sodium hydroxide and adsorption in an alumina column. The purified cobalt complex was then ashed and determined photometrically with the same reagent. In a similar procedure which Nichol (*12*) claimed was more rapid, iron was removed by a preliminary precipitation as ferric phosphate. Other impurities and excess reagent were removed from the carbon tetrachloride extract of the cobalt complex by a series of six washes with concentrated hydrochloric acid, alcoholic sodium hydroxide, and water. The purified extract was then concentrated by evaporation and cobalt measured spectrophotometrically. In the study described by Houk, Thomas, and Sherman (*5*), preliminary extractions removed iron as the chloride in isopropyl ether, and copper as its dithizonate. Cobalt was then extracted as the complex with 1-nitroso-2-naphthol in carbon tetrachloride, following which it was ashed, and finally determined spectrophotometrically with nitroso R salt. Unfortunately, this paper was mainly concerned with nutrition and gave no further chemical details. These reports from the literature represent the starting point of this investigation, the latter being preferred because of the greater specificity and colorimetric sensitivity of nitroso R salt.

In the procedure given here, excellent recoveries of microgram quantities of cobalt may be obtained routinely from bone samples as large as 25 grams, and the effect of interfering metals, a very important consideration with high-ash samples, has been greatly reduced.

APPARATUS

Spectrophotometer, Beckman, Model DU. A set of matched test tubes, 22 × 175 mm., giving an optical light path of 2.02 cm., was used in a special holder fitted to the spectrophotometer.

Glassware. Borosilicate glass Phillips beakers of 250-ml. capacity were used for wet ashing of samples; the 125-ml. size was used for wet ashing the chloroform extracts and for final color development. Extractions were made from 125-ml. borosilicate Squibb separatory funnels. All glassware was rinsed once with strong nitric acid (which may be repeatedly used), followed by tap and distilled water, to ensure thorough cleaning.

Shaking Machine. Separatory funnels were shaken about four strokes per second, 1.5-inch amplitude.

REAGENTS

All reagents were made from analytical grade chemicals.

Nitric acid, redistilled from an all-borosilicate glass still.

Methyl orange, 0.1% in distilled water.

Sodium citrate. Dissolve 500 grams of the $5^1/_2\text{H}_2\text{O}$ salt in a separatory funnel in distilled water to make almost 1 liter of solution, adjust to pH 9 with a small amount of sodium hydroxide, and extract metallic impurities with strong dithizone in chloroform until a green extract is obtained. Adjust to pH 7 with a small amount of citric acid and extract the excess dithizone from the solution with chloroform until a colorless extract is obtained. All the chloroform extracts are discarded. Separate the aqueous solution carefully from the chloroform and make to 1 liter.

1-Nitroso-2-naphthol. Dissolve thoroughly 2.5 grams of the reagent in 125 ml. of glacial acetic acid and make to 250 ml. with distilled water.

Hydrochloric acid, concentrated.

Hydrochloric acid, 1 to 99 in distilled water.

Sodium sulfate. Dissolve 10 grams of the anhydrous salt in distilled water to make 100 ml.

Phosphoric acid, 1 to 49 in distilled water.

Nitroso R salt (sodium 1-nitroso-2-hydroxy-3,6-naphthalene disulfonate), 0.1% in distilled water.

Sodium acetate. Dissolve 50 grams of the trihydrate salt in distilled water and make to 100 ml.

Standard cobalt solution, 5 γ per ml. in 1 to 49 phosphoric acid. Prepare fresh by dilution from a stronger stock solution. The latter may be made by dissolving a weighed quantity of cobalt sulfate in dilute phosphoric acid.

Chloroform.

PROCEDURE

Ashing of Samples. Either dry- or wet-ashing methods may be used for tissue and bone samples. Quantities larger than 15 grams are conveniently dry-ashed at temperatures up to 700° C. in silica dishes or in porcelain dishes with intact glaze. Samples weighing less than 15 grams are conveniently wet-ashed using nitric acid alone. Bone may be dissolved in 1 to 1 nitric acid in 1 or 2 hours on the steam bath. This solution, as well as tissue samples, may then be treated in borosilicate Phillips beakers with small portions of nitric acid. At first a low temperature is used to avoid violent flashing. After the water is driven off, the beaker is covered with a watch glass to prolong the action of the acid. When the action ceases, the beaker is cooled and the next portion is added. The temperature of the hot plate is gradually raised to full heat (400° C.) and the portions of nitric acid are reduced to the minimum required to moisten the ash completely. A very powerful oxidizing action occurs when the nitrate salts decompose at the high heat giving off oxides of nitrogen. A white ash should be obtained by either method.

Preliminary Separation of Cobalt. The ash in the original dish or beaker is dissolved in excess 1 to 1 nitric acid and evaporated to dryness in 1 to 2 hours on the steam bath, following which it is removed promptly from the heat. A hot plate may not be used for this step, as even a few degrees of heat above steam-bath temperature regenerates undesirable polyphosphate (*15*).

Tissue ash is then dissolved in 25 ml. of 1 to 49 phosphoric acid; bone ash is dissolved in 25 ml. of distilled water with the addition of 0.25-ml. portions of nitric acid and gentle heating until a clear solution is obtained. The solution is cooled and transferred to a separatory funnel. One drop of methyl orange indicator is added and the material is titrated to an orange end point (pH 3 to 4) with sodium citrate. Five milliliters of 1-nitroso-2-naphthol are added and the solution is allowed to stand for 1 hour with occasional shaking.

The cobalt complex is then extracted by shaking vigorously for 3 minutes each time with 10-ml. portions of chloroform until a colorless extract is obtained; generally this will require three portions. The extracts are combined in a second separatory funnel and impurities are removed by shaking for 2 minutes with 25 ml. of 1 to 99 hydrochloric acid. The chloroform extract is then drained into a 125-ml. Phillips beaker. Traces of cobalt remaining in the second funnel are recovered by shaking the hydrochloric acid for 1 minute with 5 ml. of chloroform, which also is drained into the beaker.

The chloroform in the purified cobalt extract is then evaporated off on the steam bath and the residue is warmed with 0.5 ml. of nitric acid until dissolved. One milliliter of sodium sulfate solution is then added as an ashing base and the mixture is evaporated to dryness on a hot plate. The beaker is covered with a watch glass, 1 ml. of nitric acid is added, and the ash is heated and agitated until dissolved and uniformly distributed over the bottom of the beaker. Heating is continued until the salts again solidify and decompose, giving off oxides of nitrogen. Ashing is completed with additional 0.5-ml. portions of nitric acid with increasing temperatures up to 400° C., in the same manner as for the original samples. A total of about 3 to 4 ml. of nitric acid is required. When a white ash is obtained, the last portion is added and the solution is examined carefully to see that it is colorless and not oily, after which the acid is evaporated off at a low heat. Any remaining color will cause an error in the final determination.

Determination of Cobalt. A clear solution should be obtained by dissolving the ash just obtained in 5 ml. of 1 to 49 phosphoric acid. To this 1 ml. of nitroso R salt reagent and 2 ml. of sodium acetate are added, with mixing after each. The solution is heated on the steam bath exactly 3 minutes; then 0.75 ml. of concentrated hydrochloric acid is added and the heating is continued for exactly 2 minutes more. (In order to adhere closely to heating times, it is convenient to use a stop watch, the samples being placed on the previously heated steam bath one at a time at 10-second intervals, so as to allow time for addition of hydrochloric acid.) The beaker is then removed and placed in a tray of cold water. After cooling, the sample is made up to exactly 10 ml. in a volumetric flask and the absorbance is read at 530 $m\mu$, using a tube containing distilled water as a reference. A blank should be run with the determination. The colors are stable for at least one day.

Standardization. Add graduated amounts up to 5 ml. of standard cobalt solution to a series of 125-ml. Phillips beakers, together with sufficient 1 to 49 phosphoric acid to make the total volume 5 ml. Develop and read the colors in the same manner as given in the preceding paragraph.

EXPERIMENTAL

Separation of Cobalt. Experiments were carried out to determine the optimal conditions for the complete extraction of cobalt. Initial attempts to extract cobalt by using a chloroform solution of 1-nitroso-2-naphthol in a manner analogous to the use of dithizone were unsuccessful. It was found that the reagent must be added in aqueous solution at the proper pH and that time must be allowed for the cobalt, normally bivalent, to be oxidized (by excess reagent) to the trivalent form required for the complex. Less time was required for the complex formation with larger amounts of 1-nitroso-2-naphthol, but more time was required when larger amounts of phosphate were present. Polyphosphates, normally formed by dehydration of phosphates during the strong heating of the ashing process, complexed cobalt and rendered it inactive toward the reagent, resulting in erratic and low recovery from bone samples. The incorporation of the nitric acid treatment step before final solution of the ash successfully hydrolyzed polyphosphate to phosphate and prevented this interference. The optimal pH for complex formation was found to be in the range of 3 to 4; poor recovery occurred at lower values, while precipitation of bone samples occurred at higher values. Sodium citrate was found to be the most convenient reagent to use for adjusting the pH without any risk of precipitation, and the amount used was not critical for proper extraction.

With the conditions which were finally developed, the contact time required for complete complexing of cobalt in a 25-gram bone sample was only 15 minutes, but 1 hour was taken as a safer and not too inconvenient period. No difficulty with precipitation in the aqueous solution was experienced. Once the chloroform extraction was begun, a slow precipitation was initiated, but the extractions easily could be completed before any appreciable precipitate accumulated. Vigorous shaking was found necessary for good extraction, especially when the separatory funnels were almost full.

Only three metals, iron, nickel, and copper, required consideration as interferences with the separation procedure. [Paulais (*14*) found that 1-nitroso-2-naphthol did not react with manganese(II), manganese(VI), manganese(VII), aluminum, lead, zinc, mercury, antimony, tin, arsenic, chromium(III), chromium(VI), and the alkali and alkaline earth metals.] The extraction of ferric iron was prevented by complexing with phosphate. The phosphate normally present in bone ash was found to be enough to suppress ferric iron; tissue ash was dissolved in phosphoric acid to achieve the same end. Ferrous iron was found to extract and occur in the acid-washed extract but the slight amount of iron in this valence which might be found in a completely ashed sample did not interfere in the final determination. No appreciable amount of nickel was found to extract under the conditions finally adopted. The copper complex in the extract was decomposed by the addition of the hydrochloric acid wash step, which also served to remove entrained salts which otherwise later would produce turbidities. Thus, the final extract which was obtained at the conclusion of the separation procedure contained a practically pure cobalt complex, together with the excess reagent.

The ashing of the chloroform extract was found to be a critical step for two reasons. First, the 1-nitroso-2-naphthol was found to be very resistant to ashing and yielded a greasy yellow solution with usual ashing reagents, such as nitric and perchloric acids. Any residual color would affect the final spectrophotometric reading. Second, cobalt was found to be lost on the glass of the ashing vessel because the extract had practically no residue. The addition of sodium sulfate was found to provide a base which prevented loss of cobalt and greatly improved the ashing action by retaining the nitric acid at higher temperatures. Nitric acid alone was found adequate for complete ashing under these conditions.

Determination of Cobalt. A number of modifications were made in the nitroso R salt procedure in order to obtain very close adherence to Beer's law even with very low values of cobalt and to improve the reliability of the determination.

Phosphoric acid was used to dissolve the ash instead of the previously reported nitric or hydrochloric acids or mixture of sulfuric and phosphoric acid (*6, 17*). Phosphoric acid alone was found to reduce the effect of interfering metals such as iron by exerting a greater complexing action and was effective in dissolving the ash.

Erratic results were avoided by a careful choice of the proper wave length for spectrophotometric reading of the color. At the wave length of maximal absorption, 418 mμ, poor results were obtained and Beer's law was found not applicable in the lower ranges (*20*). This was undoubtedly due to the fact that most of the reading was from the excess nitroso R salt, which also absorbs strongly at this wave length. McNaught (*10*) recommended reading at 540 mμ as a practical compromise. For the Beckman spectrophotometer, 530 mμ was found to be a better value, since almost half of the maximal absorption of the cobalt complex was obtained and yet the absorption of the excess reagent was negligible. This wave length was therefore adopted as the optimal one for this instrument, giving the greatest possible sensitivity with the least error attributable to slight variation in the quantity of excess reagent.

Hydrochloric acid was substituted for the generally used nitric acid in the final step of decomposing the complexes of interfering metals after color development. While nitric acid destroys about half the color of the excess reagent, it is the source of color instability (*10*). Inasmuch as the proper choice of wave length made the color of the excess reagent of no importance, better results were obtained by substituting hydrochloric acid for this step.

A steam bath was selected as being a more reproducible means of heating than the previously reported hot plate, since most of the literature indicated that the heating time in the color development steps must be closely adhered to. This precaution was taken because most of the cobalt samples were in the lower range of the method. However, it is possible that the heating times may not be critical (*13*).

With these modifications, the nitroso R salt determination was found capable of yielding excellent results. In cases where the ratio of cobalt to interfering materials is not greatly unfavorable, the separation procedure may be omitted and the determination made directly upon the ash of the sample.

Table I. Effect of Interfering Metals

Interfering Metal	Co Added, γ	Co Found, γ
Ferric iron[a], 25 mg.	0	0.0
	25	25.1
Ferrous iron, 1 mg.	0	0.1
	20	19.8
Nickel[a], 5 mg.	0	0.0
	25	25.0
Copper, 5 mg.	0	0.0
Copper, 1 mg.	25	24.4

[a] Purified reagent.

Table II. Typical Cobalt Contents Found in Normal Tissues

Tissue	Co, γ/G.
Bone, rabbit	0.03
Bone, rat	0.06
Bone, guinea pig	0.06
Kidney, rabbit	0.11
Lung, rabbit	0.03
Liver, rabbit	0.03

RESULTS

The recovery by this procedure is shown by the plots in Figure 1. When known amounts of cobalt in 1 to 49 phosphoric acid were carried through the entire procedure, including the extraction step, the color obtained was almost identical with that obtained when the standards were used directly for the final color development. The extraction efficiency was thus shown to be over 99%. The efficiency of the extraction procedure for large

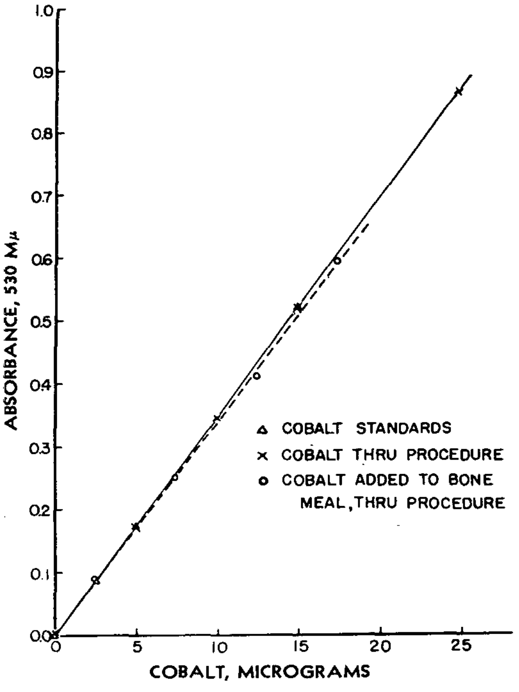

Figure 1. Recoveries of cobalt

quantities of cobalt was tested by running 1 mg. through the procedure and aliquoting the ashed solution of the extract before the final color development; recovery was 100%. The plot also shows very close adherence to Beer's law.

The recovery of the method under adverse conditions was tested using commercial steamed bone meal as a convenient material for analysis. Since this meal contained appreciable amounts of metallic iron as an impurity, it was a more severe test than ordinarily would be encountered. Known amounts of cobalt were added to 10-gram portions (equivalent in ash content to 25 grams of ordinary bone). These were dry ashed and run through the procedure, giving the results indicated in Figure 1. The amount of cobalt plotted was taken as the mean amount found in the meal (2.4 γ) plus the amount added. Average recovery was 95%. It may thus be concluded that 5% would be the maximum loss which would be encountered with bone samples up to 25 grams in size; with smaller samples the loss would be less.

The procedure showed a very high order of specificity (Table I). In order to obtain a true figure for the interference of iron and nickel it was necessary to purify the reagent grade salts of these metals: The required quantities of ferric and nickelous salts were made up in 1 to 49 phosphoric acid media and cobalt impurities were extracted exactly according to the procedure. Following the careful separation of the chloroform and evaporation of the drop floating on the surface, the aqueous residue contained the purified salts. Known amounts of cobalt were then added and the usual procedure was followed beginning with the extraction step. The purified ferric salt was used immediately to avoid slow reduction to the ferrous state. No appreciable interference occurred from quantities as large as 25 mg. of ferric iron and 5 mg. of nickel. Although ferrous iron should not normally be present in an ashed sample, tests were made with 1 mg. of the analytical grade salt (unpurified). The slight positive figure in the absence of cobalt is probably due to the cobalt impurity in the reagent; recovery of added cobalt was 99%. Copper gave no color by itself but reduced the recovery of added cobalt; however, only a 2% loss occurred with 1 mg. of this metal. Larger amounts of copper may be removed by a preliminary extraction with a chloroform solution of dithizone from the ashed solution of sample adjusted to pH 2 (*5*). Other metals do not interfere with the determination. Thus no appreciable interferences would occur in the analysis of most biologic materials.

Typical cobalt contents of normal bone and tissue samples, obtained by the procedure, are given in Table II.

ACKNOWLEDGMENT

The author is indebted to F. L. Hyslop for assistance with the literature search and to D. H. Byers and H. E. Stokinger, under whose direction the work was carried out, for their valuable criticism and review.

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RECEIVED for review August 11, 1954. Accepted November 1, 1954.

Separation of Alpha-Keto Acid Dinitrophenylhydrazones by Paper Electrophoresis and Their Colorimetric Determination

HENRY TAUBER

U. S. Public Health Service, University of North Carolina, Chapel Hill, N. C.

This report describes a convenient procedure for the separation and quantitative determination of pyruvic acid and α-ketoglutaric acid. These keto acids are isolated in pure state as the 2,4-dinitrophenylhydrazones and separated by filter paper electrophoresis. The separated bands are extracted with sodium hydroxide and the color intensities are measured colorimetrically. The procedure has been applied to the analysis of different mammalian bloods.

KETO acids form a link between carbohydrate and protein metabolism. Each keto acid has its specific function. Thus, the determination of the total keto acids in biological materials as practiced in the past is of limited value. A suitable and rapid procedure for the quantitative determination of each of these compounds is of biochemical interest.

Recently several investigators (*1–4, 8*) recommended methods for the determination of pyruvic acid and α-ketoglutaric acid based on the chromatographic separation of their 2,4-dinitrophenylhydrazones. In all of these chromatographic methods the hydrazones are separated on large sheets of filter paper with the aid of a combination of solvents and salts. The procedure requires at least 24 hours. Under those conditions pure pyruvic acid separates into two or three spots (*1–4, 8*). It has been suggested that these spots are the cis-trans isomers and a polymer of the pyruvic acid hydrazone. Paper chromatography, based on this procedure, is not the ideal method for the analysis of α-keto acids.

There is described here an application of filter paper electrophoresis to the separation of the 2,4-dinitrophenylhydrazones of α-ketoglutaric acid and pyruvic acid. The separated bands are extracted with sodium hydroxide and their color intensities are determined colorimetrically. Commercially obtainable apparatus are employed. The procedure has been applied to the analysis of protein-free blood filtrates.

Because the major α-keto acid constituents of the blood are pyruvic acid and α-ketoglutaric acid (the other keto acids are seldom seen and then only in traces), this report deals only with these two keto acids. However, there is a hydrazone obtainable from protein-free filtrates of mammalian blood which has a mobility very close to that of α-ketoglutaric acid hydrazone but different characteristics.

The present investigation was well in progress when the interesting paper by Neish (*6*) on the separation of α-keto acid dinitrophenylhydrazones and their polarographic estimation came to the author's attention. Some of his separation experiments have been repeated and extended and a comparative study of the blood of several mammals was made. The polarographic method in which the nitro compounds are reduced in acid solution permits the measurement of very small quantities of keto acids. According to Neish (*6*), however, his method is not completely foolproof. The polarographic method was not investigated in this work.

EXPERIMENTAL

Solutions. 6*N* hydrochloric acid; 1*M* sodium carbonate; 1*N* sodium hydroxide; ethyl acetate, C.P.; 400 mg. of 2,4-dinitrophenylhydrazine dissolved in 40 ml. of 95% ethyl alcohol and 10 ml. of concentrated hydrochloric acid, and diluted to 200 ml. with 95% ethyl alcohol; 10% sodium tungstate; 0.67*N* sulfuric acid; barbital buffer of 0.1 ionic strength [55.8 grams of sodium barbital, 38.8 grams of sodium acetate ($3H_2O$), 5900 ml. of distilled water, and about 48 ml. of 1*N* hydrochloric acid to make a final pH of 8.5; the final volume is made up to 6000 ml.].

Stopcock grease (Nonaq, Fisher Scientific Co.) that is insoluble in organic solvents was employed. Ordinary stopcock grease is readily carried over during the final extraction of the hydrazones with ether and the grease retains a small quantity of the hydrazones on the line of application.

Preparation of Pure Hydrazones. Purified crystalline sodium pyruvate was used in these investigations (*7*). The α-ketoglutaric acid (Organic Specialties, Inc.) was not further purified. Both compounds were found to be pure by paper electrophoresis. Stock solutions of the keto acids containing 10 mg. of the free acids per 10 ml. of distilled water were prepared daily, and were further diluted with distilled water to the desired concentrations. The hydrazones were purified and isolated by the conventional procedure.

To 100 ml. of keto acid solution containing 100 to 1500 γ of keto acid or mixtures of keto acids, 5 ml. of 2,4-dinitrophenylhydrazine were added. The mixture was kept for 30 minutes at 25° C. It was then put into a 250-ml. separatory funnel and the hydrazones were extracted three times with 20-ml. portions of ethyl acetate. Emulsions, if formed, were separated by centrifuging for a few minutes. The combined ethyl acetate layers were extracted twice with 30-ml. portions of 1*M* sodium carbonate. The combined sodium carbonate layers were washed twice with 20-ml. portions of ethyl acetate, transferred to an Erlenmeyer flask, and cooled in an ice bath. Then the cold carbonate solution was acidified with 20 ml. of cold 6*N* hydrochloric acid. The acid was added dropwise while the flask was rotated in order to avoid accumulation of excess acid. This precaution eliminated the formation of isomers and polymers (*1–4, 6, 8*). The hydrazones were extracted from the acidified solution with two 20-ml. portions of ether. The ether extract was dried with anhydrous sodium sulfate and then decanted into a test tube. The sodium sulfate was washed with 2 ml. of ether. The ether extracts were combined and the ether was removed by immersing the tube into hot water. Final traces of solvent were removed in vacuo. The dry 2,4-dinitrophenylhydrazones were dissolved in 0.2 to 0.4 ml. of ethyl alcohol. The blood keto acid hydrazones were dissolved in about 0.1 ml. of ethyl alcohol.

Paper Electrophoresis. One of the apparatus employed in this work was that of Biophysical Instruments, Inc., Philadelphia, Pa. It operated close to 150 volts and 11 ma. Two determinations were made simultaneously using 285 × 65 mm. Whatman No. 1 filter paper strips. One hundred microliters of hydrazone solution, equal to 10 ml. of blood, were applied on a line with a capillary pipet, 80 mm. from one end of the strip. The other instrument employed was that of Grassmann and Hannig (E. Miltenberg, Inc., New York, N. Y.). It operated close to 110 volts and 16 ma. For this apparatus, however, it was necessary to construct a special frame or rack holding one 285 × 80 mm. strip. The original frame was devised to hold two narrow strips. The parts for the new frame were cut from Plexiglas and annealed with acetone. As this apparatus permits the use of three chambers, three determinations were made simultaneously.

Prior to the application of the hydrazones the filter paper strips were saturated with barbiturate buffer of pH 8.45. Neish (*6*) employed 0.05*M* sodium carbonate as the electrolyte. Barbiturate buffer, however, offers neater separation and narrower bands. Excess buffer was removed by blotting the strips with sheets of (20 × 20 inches) Eaten-Dickman filter paper No. 320. These sheets were found to be very convenient because of their great absorbing capacity. The sheets were re-used many times, as, owing to their unusual thickness (3 mm.), they are durable. The electrophoresis was conducted at 23° C. in a temperature- and humidity-controlled room. This precaution, however, was necessary only during unusually high summer temperatures.

Pure pyruvic acid and α-ketoglutaric acid hydrazones were separated in 2 to 3 hours. Blood pyruvic acid and α-ketoglutaric hydrazones were separated in 4 to 5 hours. Then the frames containing the strips were removed and dried at 40° C. in an oven with circulating air supply.

After the strips have been dried the yellow colored bands were cut out, sliced, and placed in centrifuge tubes. The yellow center line or bar which often separated between the pyruvic acid and α-ketoglutaric acid hydrazone bars and which appeared close to the α-ketoglutaric acid hydrazone bar was always included with α-ketoglutaric acid hydrazone (see discussion of component X). Six milliliters of 1*M* sodium hydroxide were added to the tube containing α-ketoglutaric acid hydrazone shreds and 12 ml.

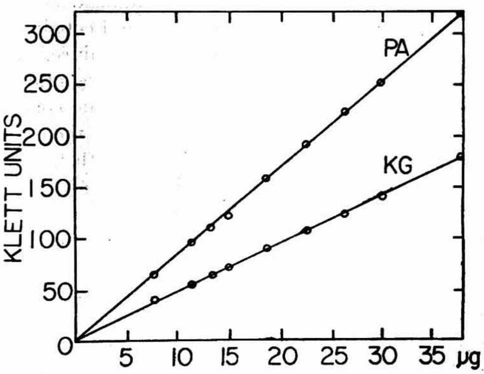

Figure 1. Calibration curves of hydrazones of pyruvic acid and α-ketoglutaric acid after separation by paper electrophoresis

Electrolyte, barbiturate buffer, pH 8.45
Time, 3 hours
Temperature, 23° C.

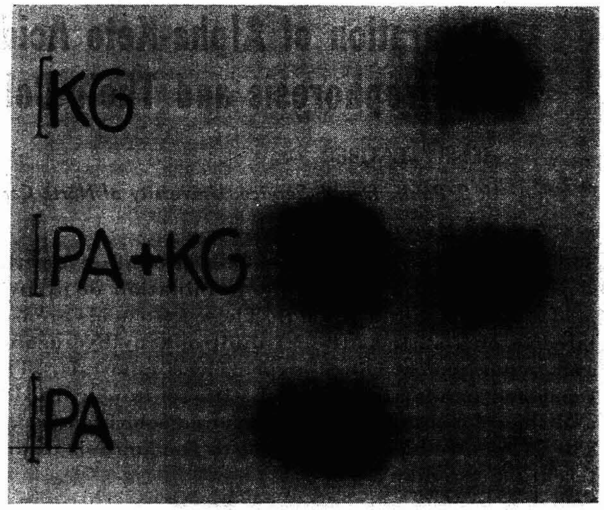

Figure 2. Electrophoretic pattern showing spots for 2,4-dinitrophenylhydrazones of α-ketoglutaric acid (*KG*), a mixture of hydrazones of pyruvic acid and α-ketoglutaric acid (*PA* + *KG*), and of pyruvic acid (*PA*)

There were 4 γ of each keto acid (on free acid basis) in 2 µl. of ethyl alcohol
Markings indicate lines of application
Electrolyte, barbiturate of pH 8.45
Time, 3 hours
Temperature, 23° C.

(24 ml. or more when other than human blood was analyzed) to the tube containing pyruvic acid hydrazone shreds. The tubes were stoppered with a rubber stopper, shaken for 2 minutes, and centrifuged for 10 minutes. The supernatants were placed in Klett tubes. If filter fibers were present, the tubes were recentrifuged. The color intensities were measured, without further decanting, in a Klett-Sumerson photoelectric colorimeter using green filter No. 54. The keto acid values were obtained from the calibration curves (Figure 1).

Separation of Pure Pyruvic Acid and α-Ketoglutaric Acid Hydrazones. Figure 2 shows a filter paper strip where the hydrazone of 4 γ of pyruvic acid in 2 μl. of ethyl alcohol was applied to a 285 × 80 mm. strip (uppermost spot). In the second row a mixture of the hydrazones of 4 γ of pyruvic acid and 4 γ of α-ketoglutaric acid was applied; and in the lowest spot the hydrazone of 4 γ of α-ketoglutaric acid was applied to the strip and subjected to paper electrophoresis for 3 hours. It is seen that the mobility of α-ketoglutaric acid hydrazone is greater than that of pyruvic acid hydrazone. The separation of pyruvic acid hydrazone from α-ketoglutaric acid hydrazone is distinct. The hydrazones were freshly prepared. They moved anodeward.

In Figure 1 are shown calibration curves obtained when mixtures (7.5 to 38.0 γ) of pyruvic acid and α-ketoglutaric acid were converted to their 2,4-dinitrophenylhydrazones and subjected to paper electrophoresis. The different concentrations of hydrazone solutions were obtained by diluting a stock solution of the two hydrazones with ethyl alcohol. The graph shows that the hydrazone of pyruvic acid gives a much more intense color with $1N$ sodium hydroxide than the hydrazone of α-ketoglutaric acid. Separation of the two hydrazones appears to be quantitative. There is a proportionality between color intensity and hydrazone concentration in accordance with Beer's law. Figure 2 was obtained by spraying the strips, after electrophoresis and drying, with $1N$ sodium hydroxide using a glass sprayer (Research Equipment Corp.) attached to a Gast rotary pump. Here separation is distinct. Pyruvic acid hydrazone solutions should not be kept for too many hours at room temperature; otherwise isomerization and polymerization occurs (*1–4, 6, 8*).

Application to Blood Analysis. Freshly drawn blood (15 ml.) was added at once to 105 ml. of water and 15 ml. of $0.66N$ sulfuric acid. This was mixed and 15 ml. of 10% sodium tungstate were added. After shaking for 1 minute, the mixture was kept for 10 minutes at room temperature, then centrifuged for 7 minutes in a refrigerated centrifuge at 5° C. If desired, an ordinary centrifuge may be used. The supernatant was filtered through a small folded filter paper. In the recovery experiments the standard was added to the whole blood. Human blood was taken from the antecubital vein (laboratory personnel), sheep blood from the jugular vein, and rabbit and guinea pig blood was obtained by cardiac puncture. All bloods were taken under nonfasting conditions.

To 100 ml. of blood filtrate 5 ml. of 2,4-dinitrophenylhydrazine were added and mixture was treated similar to the pure keto acids. The dry hydrazone residues were dissolved in 80 microliters of ethyl alcohol. This was applied to the strip. The test tube was "rinsed" with 20 μl. of ethyl alcohol and this portion too was applied to the strip.

In Table I are recorded pyruvic acid values for rabbit, sheep, guinea pig, and human blood. Neish's values for rat blood (*6*) are included for comparison. Rabbit blood contains several times more pyruvic acid than the blood of other mammals. Differences in α-ketoglutaric acid values (Table II) are not as marked except for guinea pig blood, which contains very small quantities of α-ketoglutaric acid.

The recovery of added pyruvic acid to rabbit blood (see typical results in Table I) ranges from 96 to 110%, with a mean of 99%. The recovery of α-ketoglutaric acid (Table II) ranges from 81 to 85%, with a mean of 83%. When mixtures of pure pyruvic acid and α-ketoglutaric acid were analyzed the recovery was 90 to 95%.

Table I. Pyruvic Acid in Different Mammalian Bloods

(γ per 10 ml. of blood)

Type of Mammal	1	2	3	4	5	6	Mean
Rabbit	288	216	216	200	188	296	234.0
Plus 100 γ pyruvic acid	376	296	326	288	296	392	...
Recovery, %	97	94	110	88	108	96	98.8
Human	98	70	71	76	84	74	78.8
Sheep	176	188	122	128	110	150	145.7
Guinea pig	50	128	80	72	90	142	93.7
Rat (*6*)	118	121	126	66	59	79	94.8

Table II. α-Ketoglutaric Acid in Different Mammalian Bloods

(γ per 10 ml. of blood)

Type of Mammal	1	2	3	4	5	6	Mean
Rabbit	54	50	52	36	23	51	44.3
Plus 100 γ α-ketoglutaric acid	132	128	118	118	107	132	
Recovery, %	85	85	82	82	84	81	83.2
Human	21	20	17	25	20	14	19.5
Sheep	40	57	38	40	34	76	47.5
Guinea pig[a]	8	11	10	6	7	13	9.2
Rat (*6*)	48	33	13	22	18	51	30.8

[a] Here, because of the unusually low α-ketoglutaric acid content, 25 ml. of blood were employed in the analysis. The values, however, are per 10 ml.

Component X. Often a band appears between the pyruvic acid and the α-ketoglutaric acid hydrazone bands when mammalian blood hydrazones are separated on filter paper. This band between pyruvic acid and α-ketoglutaric acid has been observed by Neish (*6*) in rat liver extracts but not in extracts of other rat tissue or rat blood. While the present paper was in press, Neish (*5*), advised this author that he also observed component X in some rat blood filtrates. He has named this band Spot X (*6*). It was observed in the present work that when the dried strips were sprayed with $1N$ sodium hydroxide spot X gave a light yellow color as opposed to the usual dark red-brown and dark brown color of pyruvic acid and α-ketogutaric acid hydrazones. Whenever the center band X did not separate, its presence was identified as follows: The α-ketoglutaric acid bar was cut in the middle and immersed in $1N$ sodium hydroxide; the slower moving half of the bar gave a yellow color, whereas the faster moving α-ketoglutaric acid hydrazone bar gave a dark brown color. Component X is probably not an isomer of pyruvic acid or α-ketoglutaric acid hydrazone. If green filter No. 54 is used, the Klett reading of this component is negligible. Component X does not appear when pure hydrazones are subjected to paper electrophoresis.

Neish (*6*) did not notice this marked difference between the keto acid hydrazones and component X because by using the polarographic method he worked in an acid medium in which all hydrazones were yellow. He included spot X in the α-ketoglutaric acid hydrogen spot. The identity of component X is not known at present. 2,4-Dinitrophenylhydrazine reagent gives a grayish brown color with $1N$ sodium hydroxide. This reagent, however, is removed during the purification of the 2,4-dinitrophenylhydrazones. The bands of pyruvic acid and α-ketoglutaric acid as separated by the present method may include traces of other keto acid hydrazones.

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RECEIVED for review July 29, 1954. Accepted October 29, 1954.

Technique for Continuous Intensity Standardization in Quantitative Analysis by Nuclear Magnetic Absorption

R. H. ELSKEN and T. M. SHAW

Western Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Albany 6, Calif.

A technique for nuclear magnetic absorption measurements provides for continuous comparison of the absorption signal with a reference standard. A Watkins-Pound calibrator circuit serves as a source of the reference signal. A servo-system is used to compare continuously the magnitudes of the reference and absorption signals. The method facilitates accurate determination of absorption line intensity.

WITH expanding interest in analytical applications of magnetic resonance spectroscopy, there has developed a need for precise measurements of line intensity. The present communication is concerned with a method developed for this purpose. Measurement of line intensity involves the stabilization of spectrometer circuits and components to the degree of accuracy required, or the use of a standard of comparison. In the initial consideration of the problem of using a spectrometer for intensity measurements, a method was sought for providing a reference standard of intensity. Standard samples have proved only partially satisfactory, principally because of short time fluctuations in spectrometer operating conditions and changes in sample environment when switching to the standard. It is possible to use a double coil arrangement or to combine a standard with the material being examined and thus reduce these effects. This paper, however, is concerned with another method more satisfactory for present purposes, which utilizes an electronic standard of reference (*4, 5*) in a manner so as to provide continuous comparison of the absorption signal with the standard. The method has been found useful in an application of proton resonance absorption for measurement of moisture in hygroscopic solids (*2, 3*). The technique should be of interest in other applications of magnetic resonance absorption.

THEORY

Use of the absorption signal with present equipment (*1*) involves detection of small changes in input conductance G of a tuned circuit in a two-terminal negative resistance oscillator. In the presence of an absorption, G is modulated at the static field angular modulation frequency, ω_m. The oscillator is particularly sensitive to changes in tuned circuit conductance, and a small increase in conductance causes a large decrease in oscillation level. The calibrator unit supplies an "artificial" absorption in the form of a modulated electronic resistance connected across the coil containing the sample, and produces a known change in conductance ΔG_{calib} at the spectrometer radio-frequency oscillator tuned circuit. This is analogous to the change in tuned circuit conductance $\Delta G_{\text{specimen}}$ caused by the absorption of *r-f* energy from the spectrometer by the sample.

The spectrometer unit is operated so that the specimen signal is detected as a sinusoidal variation. The static magnetic field is varied slightly about a fixed value by applying a sinusoidal voltage of frequency, f_m, to a pair of coils wound on the permanent magnet pole pieces. With the resulting static field fluctuation less than the line width of the resonance curve, the spectrometer output in the region of resonance is a sinusoidal voltage whose amplitude depends on the slope of the absorption curve at a specific operating point. This operating point is moved across the region of absorption by changing the *r-f* oscillator frequency. Effects of wave-form distortion caused by nonlinearity in absorption curve slope are reduced by a narrow band audio amplifier following the oscillator unit output. The calibrator input signal and resulting artificial absorption signal are also sinusoidal variations.

With the calibrator voltage originating from the same source or synchronized with the static field modulation voltage, the artificial absorption signal can be adjusted for any fixed phase relative to the nuclear magnetic resonance signal. It is a 0° or 180° phase relationship when a lock-in detector is employed if the detector is to respond to both the true and artificial signal.

For use of the calibrator reference signal without the null-balance feature, a specimen run is made and recorded. Immediately preceding or following this a calibrator standard signal is recorded. It is proposed in the null-balance system that the specimen signal and calibrator signal be presented at the same time, and that the phase of the calibrator signal be adjusted so that it is 180° out of phase with the specimen signal. Since the calibrator signal is introduced at the same point in the oscillator unit as the specimen signal, addition of the two signals occurs at the coil containing the specimen. A calibration signal amplitude then can be chosen so that the vector addition of the two signals results in a null-balance condition at the oscillator, a zero change in total conductance, G, of the tuned circuit and zero signal output from the oscillator unit as long as ΔG of the tuned circuit due to the calibrator influence is equal to and 180° out of phase with ΔG of the tuned circuit due to the influence of the specimen energy absorption. Then spectrometer output or error voltage is proportional to ΔG_{total}.

$$S \alpha \Delta G_{\text{total}} = [\Delta G_{\text{specimen}} \sin \omega t] + [\Delta G_{\text{calib.}} \sin (\omega t + 180°)]$$

where

$$\omega t = \omega_m t - \phi$$

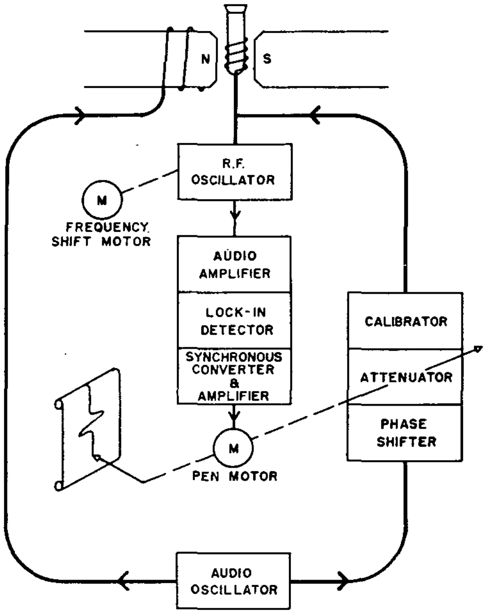

Figure 1. Block diagram of nuclear resonance spectrometer

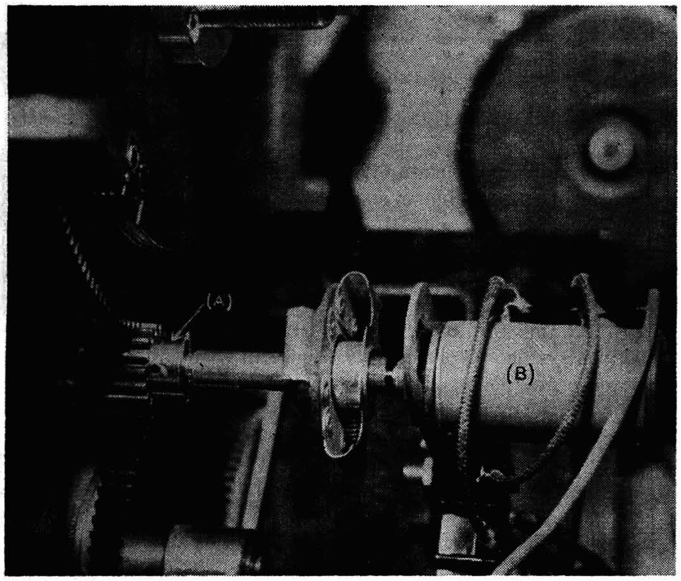

Figure 2. Recorder pen motor coupling to calibrator unit input attenuator

A. Pen motor shaft
B. Helical potentiometer

Watkins (*4*) calls attention to a phase lag ϕ between the amplitude modulation of the *r-f* oscillator and the modulating signal, which may vary from 0° to 90°, depending upon the oscillation level and the values of circuit parameters. This phase lag then may vary with changes in samples, as, for example, if there are changes in the dielectric properties of the samples influencing the total tuned circuit conductance. As a result, when using the spectrometer without the null-balance feature, the phase of the lock-in voltage should be readjusted for each new operating condition. The lock-in detector is a phase sensitive device, and the output depends on the relative phase between the input voltage and the reference or lock-in voltage which is synchronized with the modulation voltage. However, with the above change in ϕ applying to the calibrator also, and operating the calibrator in a continuous balance system, the effects of the phase shift will not be so evident since the change operates only upon the net error signal, S. Effects of the phase change will not be evident until the output of the lock-in detector is insufficient to maintain normal operation.

With the null-balance system there is a possibility of self-compensation for small fluctuations in the audio oscillator output, which is the source of static magnetic field modulation and calibrator input voltage. With linear response in both the modulation circuit and calibrator circuit a small change in audio oscillator output would have no effect past the point of addition of the two signals, since the total voltage gain in the modulation loop equals the total voltage gain in the calibrator loop for null conditions at the sample coil.

The calibration signal amplitude thus can be used as a continuous measure of the nuclear magnetic resonance absorption signal amplitude.

EXPERIMENTAL

The nuclear resonance spectrometer and the system for maintaining this continuous zero balance can be represented basically in block form as shown in Figure 1. The general circuit arrangement is conventional with the exception of the recorder pen motor coupling to the calibrator input voltage attenuator. The net error signal, S, is fed to a narrow band amplifier and to the lock-in detector. The output of the lock-in detector, which is a direct current voltage, is fed directly to a synchronous converter, amplified, and used to drive a motor connected to the pen mechanism of a strip chart recorder. The synchronous converter and amplifier is the chopper amplifier normally supplied with the recorder, modified by disconnecting the potentiometer circuit and feeding the input directly to the chopper. In this condition the pen motor will continue to rotate as long as a voltage is present at the input to the recorder, or until the stops at the chart extremities are reached. In the photograph, Figure 2, it can be seen that the pen motor is also directly coupled to the shaft of a helical potentiometer which is the amplitude control at the input to the calibrator unit. This potentiometer is of the ten-turn type which corresponds closely to the number of revolutions required of the Brown recorder pen motor to move the pen mechanism full scale on the chart. Although not investigated by the authors, an equivalent coupling may be applied to other recorders. This attenuator coupling completes a feedback loop through the calibrator back to the sample coil connection.

When the specimen absorption signal amplitude changes as the spectrometer frequency is moved through the absorption region and makes $S > 0$, the error voltage is detected and amplified which causes the pen motor to rotate the helical potentiometer until the calibrator signal and specimen signal are again equal. The only function of the spectrometer amplifier is to provide sufficient error voltage to operate the pen motor. Amplifier amplitude stability should be of minor importance in this operation. The calibrator input impedance is approximately 100 times the helical potentiometer impedance so the potentiometer input is linear with rotation. The pen trace for the recorder which is proportional to the calibrator voltage is then a reproduction of the nuclear resonance line.

Since the specimen signal obtained with the spectrometer is a derivative of the absorption line, a means must be provided for phase reversal of the calibrator output as the specimen signal goes through the maximum absorption portion of the line where the slope of the curve is zero. An audio-transformer with grounded center tap in the secondary supplies the calibrator attentuator with an input balanced to ground. This provides center scale zero for the potentiometer and recorder pen and accomplishes the necessary phase reversal either side of zero.

It is necessary to provide adequate controls and checks of calibrator unit performance. A method of accomplishing this is to meter the calibrator input voltage and calibrator plate current. A primary standard sample, for calibrator calibration, may also be necessary for checking operation from time to time.

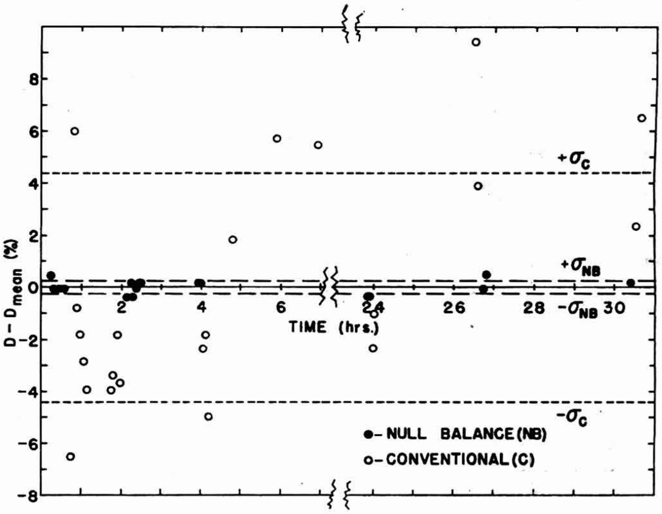

Figure 3. Test results on output stability of conventional system and null-balance system

Voltage stabilization removed from radio-frequency spectrometer narrow band audio-amplifier

The time constant of the complete loop must not greatly exceed the response time of the pen and helical potentiometer drive motor or severe hunting will result.

PERFORMANCE

In order to determine the performance of the null-balance system relative to that of the conventional system without the calibrator feedback loop, an instrument was set up, and a number of tests were performed. As a test material an aqueous 0.15*M* solution of ferric nitrate was used.

Complete absorption line derivatives were obtained with the null-balance system and the conventional system. Plots of the rate of change of magnetic absorption with frequency versus *r-f* oscillator frequency were obtained. A detailed comparison by superposition showed the identical shape of the two lines. This test indicated that the "electronic" standard functions in accord with the simple theory and does not introduce errors in line shape.

Since the primary function of the spectrometer amplifiers is to provide sufficient error voltage to operate the pen and helical potentiometer motor in the null-balance system, tests were conducted to determine the effect of gain variations on the system. Deliberate changes in spectrometer audio amplifier gain were introduced and the peak-to-peak amplitude of the recorded derivative was measured for the various gain conditions. A maximum variation of ± 0.43% in the average derivative was recorded for a gain variation of ± 18% from normal. This is approximately the limit of precision that can be expected of the present equipment.

A more realistic approach for checking the effects of circuit instability on the results obtained with the null-balance system was obtained by disabling the voltage stabilization circuit of the spectrometer narrow band audio amplifier. Figure 3 shows the variations in peak-to-peak amplitude of the recorded line for the null-balance system compared with results for the conventional system, both operating under the above conditions. Readings were taken on a single specimen that remained untouched beginning approximately 10 minutes after the equipment was turned on from a cold start and extending over a period of 30 hours. Equipment was alternated between conventional operation and null-balance operation during this test period with a minimum of disturbance and under controllable conditions. The standard deviation over a period of 30 hours was ± 0.3% for the null-balance system as compared with ± 4.4% for the conventional system.

A source of difficulty encountered with the spectrometer operated conventionally is the long time required for the equipment to come to temperature equilibrium. With the conventional operation several hours warm-up time is required before the instrument readings become stable, even with stabilized amplifiers. Twenty minutes after start with the null-balance system, readings were consistent within ± 0.3%. For the conventional system after a 1-hour warm-up the average amplitude had yet to reach a stable level.

In addition to quantitative moisture measurements, the null-balance system at present is being used to facilitate relaxation time measurements, or situations where a change in spectrometer radio-frequency energy level in the specimen coil is desired while holding the system gain constant.

On the basis of performance to date, the system appears to provide performance of the type essential for precision amplitude measurements. It permits amplitude measurements utilizing amplifiers that do not have to be stabilized to a high degree, provides a continuous check of system gain with the specimen in place and in its normal environment, and warm-up time is considerably reduced where time is an important element in operation.

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RECEIVED for review August 10, 1954. Accepted October 2, 1954.

Nonaqueous Titration Method for Determination of the Purity of Hexahydro-1,3,5-trinitro-*s*-triazine

And Its Content in Wax and Polyisobutylene—Motor Oil Compositions

SEYMOUR M. KAYE
Picatinny Arsenal, Dover, N. J.

A rapid, reliable method was needed for the determination of the purity of hexahydro-1,3,5-trinitro-s-triazine (RDX) and its content in explosive compositions. Its purity and amount present in composition with wax and polyisobutylene–motor oil were determined by titration in a dimethylformamide medium using a solution of sodium methoxide in benzene-methanol as titrant. The end point of the titration is obtained visually, using azo violet as indicator.

THE determination of the purity of hexahydro-1,3,5-trinitro-*s*-triazine (RDX, hexogen) has long been troublesome to analysts of explosives. Several attempts have been made through the years to establish a satisfactory method. In 1921 Rathsburg (*6*) first used titanous chloride to determine the "nitration product of hexamethylenetetramine," but he neither balanced the equation nor identified the reaction product. He suggested the equation:

$$C_3H_6N_3(NO_2)_3 + 12\ TiCl_3 \rightarrow C_3H_6N_6$$

Desvergnes (*2*) attempted to determine it by breaking it down to nitrogen in a nitrometer, but conceded failure because less than five sixths of its total nitrogen was liberated in elemental form. A later attempt to reduce hexahydro-1,3,5-trinitro-*s*-triazine with titanous chloride solution (*5*) succeeded only to the extent of about 60%, even on prolonged boiling. The use of ferrous chloride effected a negligible reduction. When both reducing agents were added to the same sample, strangely enough, the reduction was over 90% complete. By use of a 300% excess of titanous chloride, 20 ml. of 0.7*N* ferrous chloride, and a 30-minute boiling period, the reduction was made to proceed to 98 to 99% of the theoretical. This method, however, was completely empirical, with even the slightest deviation from the above conditions resulting in erratic results. A characteristic color reaction with sodium nitroferricyanide has been utilized for a spectrophotometric method of analysis (*10*). A maximum absorbance for the color system is obtained at a wave length of 625 mμ. Although the system does not conform to Beer's law, a linear relationship exists between transmittance and concentration in the range of 100 to 200 p.p.m. This method, too, is highly empirical, and requires very critical experimental conditions. Identifying physical properties such as spectrophotometric data (*4*, *7*) and x-ray diffraction patterns (*8*), as well as related chemical work (*1*, *9*), has been reported.

Fritz and Lisicki (*3*) have demonstrated that many types of organic compounds can be determined by titration as acids in suitable organic solvents, and have recommended both the use of sodium methoxide in benzene-methanol as titrant and dimethylformamide as solvent. Dimethylformamide (DMF) was chosen as the solvent because of the ready solubility of hexahydro-1,3,5-trinitro-*s*-triazine and the fact that it is commercially available at a reasonable price and requires no further purification prior to use.

The nonaqueous titration method described in this report has been applied to samples of the purified product and synthetic samples of composition A-3 (91% RDX, 9% wax desensitizer) and composition C-4 (91% RDX, 9% polyisobutylene–motor oil binder).

Table I. Analysis of Samples of Purified RDX

Sample No.	RDX Found, %	Error, %	Deviation from Average Found, %
1	99.70	0.30	0.18
2	100.06	0.06	0.18
3	99.90	0.10	0.02
4	100.02	0.02	0.14
5	99.81	0.19	0.07
6	99.90	0.10	0.02
7	99.77	0.23	0.11
Average	99.88	0.14	0.10

Added, %	100.00
Found average, %	99.88
Standard deviation[a], %	0.13
Error, %	0.14

[a] Obtained by dividing the summation of the squares of the individual deviations by a number equal to one less than the number of determinations and taking the square root of the quotient.

Table II. Analysis of Synthetic Samples of Composition A-3

Sample No.	RDX Found, %	Error, %	Deviation from Average Found, %
1	91.07	0.07	0.09
2	90.80	0.20	0.18
3	91.12	0.12	0.14
4	90.96	0.04	0.02
5	90.84	0.16	0.14
6	91.18	0.18	0.20
7	90.90	0.10	0.08
Average	90.98	0.12	0.12

Added, %	91.00
Found average, %	90.98
Standard deviation, %	0.15
Error, %	0.12

Table III. Analysis of Synthetic Samples of Composition C-4

Sample No.	RDX Found, %	Error, %	Deviation from Average Found, %
1	90.90	0.10	0.07
2	90.88	0.12	0.09
3	91.04	0.04	0.07
4	91.09	0.09	0.12
5	90.94	0.06	0.03
6	90.84	0.16	0.13
7	91.10	0.10	0.13
Average	90.97	0.14	0.09

Added, %	91.00
Found average, %	90.97
Standard deviation, %	0.12
Error, %	0.14

EXPERIMENTAL PROCEDURE

Preparation of Reagents. Azo Violet Indicator. Prepare a saturated solution of 1,4-nitrobenzeneazoresorcinol in benzene.

Sodium methoxide solution, 0.1*N*. Add 100 ml. of absolute methanol to a 2-liter borosilicate glass storage bottle. Wash approximately 5 grams of freshly cut sodium metal with absolute methanol and add piecemeal to the 2-liter bottle. The reaction of sodium with the methanol can be controlled by occasional immersion of the reaction vessel in ice water. When the reaction is complete, add 150 ml. of absolute methanol and 1500 ml. of benzene, C.P. grade, and store protected from carbon dioxide and moisture. Standardize against benzoic acid, National Bureau of Standards grade, with the aid of azo violet indicator as follows:

Transfer 50 ml. of dimethylformamide, technical grade, to a 250-ml. beaker and add 4 to 5 drops of azo violet indicator solution. Cover the beaker with a glass cover containing a hole in the center to admit a buret tip, and titrate to a clear blue color with 0.1*N* sodium methoxide solution, employing a magnetic stirrer. Transfer an accurately weighed portion of approximately 0.5 gram of the benzoic acid to the 250-ml. beaker. Titrate with 0.1*N* sodium methoxide to the clear blue color. Calculate the normality of the sodium methoxide solution as follows:

$$\text{Normality of sodium methoxide solution} = \frac{W}{0.1221V}$$

where W = weight of benzoic acid and V = volume of 0.1*N* sodium methoxide solution used in the titration after the dimethylformamide has been neutralized.

Although the reagent is reasonably stable, it should be restandardized every few days.

Determination of Purity and Content of Composition A-3. Transfer 50 ml. of dimethylformamide, technical grade, to a 250-ml. beaker and add 4 to 5 drops of azo violet indicator solution. Cover the beaker with a glass cover containing a hole in the center to admit a buret tip, and titrate to a clear blue color with 0.1*N* sodium methoxide solution, employing a magnetic stirrer. Transfer an accurately weighed portion of approximately 0.3 gram of dry sample to the beaker. Titrate with 0.1*N* sodium methoxide solution to a green end point which persists for 30 seconds. Calculate to purity or percentage of hexahydro-1,3,5-trinitro-*s*-triazine in composition A-3 as follows:

$$\text{Purity or percentage of RDX in sample, \%} = \frac{7.40\ VN}{W}$$

where

V = volume of 0.1*N* sodium methoxide solution used in titration after dimethylformamide has been neutralized
N = normality of 0.1*N* sodium methoxide solution
W = weight of sample on a moisture-free basis

Determination of Content of Composition C-4. Transfer an accurately weighed portion of approximately 1.5 grams of dried sample to a 150-ml. beaker. Add 25 ml. of carbon tetrachloride, transfer beaker and contents to a hot plate, and swirl until all of the binder is dissolved as evidenced by the complete separation of hexahydro-1,3,5-trinitro-*s*-triazine crystals. Remove the beaker and contents from the hot plate and cool to room temperature. Use a fine porosity filter stick and suction to remove all of the carbon tetrachloride. Add 50 ml. of dimethylformamide, technical grade, to the beaker and stir the mixture using the filter stick until all of the RDX is dissolved. Transfer the solution quantitatively to a 100-ml. volumetric flask, and make up to volume with dimethylformamide. Prepare a blank using dimethylformamide so that the blank is handled in exactly the same manner as the sample. Withdraw a 20-ml. aliquot by pipet and transfer to a 100-ml. tall-form beaker containing 5 drops of azo violet indicator solution and a magnetic stirrer. Cover the beaker with a glass cover containing a hole in the center to admit a buret tip, and titrate with 0.1*N* sodium methoxide to a green end point which persists for 30 seconds. Employ the magnetic stirrer in the course of the titration. Repeat the titration procedure for the blank. Calculate to percentage of RDX in composition C-4 as follows:

$$\text{RDX, \%} = \frac{(A - B)\,(7.40)\,(N)}{W}$$

where

A = volume of 0.1*N* sodium methoxide used for sample
B = volume of 0.1*N* sodium methoxide used for blank
N = normality of 0.1*N* sodium
W = weight of sample on a moisture-free basis represented by aliquot taken

DISCUSSION

The purified product employed in the methods described above was prepared by recrystallizing U. S. Government specification grade Type A material [no octahydro-1,3,5,7-tetranitro-s-tetrazine (HMX) present] from acetone until a corrected melting point of 201° C. was obtained.

In the original survey of the problem of titrating composition C-4, several approaches were attempted with little success. One method involved the direct titration of composition C-4 in dimethylformamide. This resulted in low, erratic results characterized by drifting, indefinite end points. These were undoubtedly due to the occlusion of hexahydro-1,3,5-trinitro-s-triazine crystals by the polyisobutylene binder, which prevented complete solution in dimethylformamide. A second approach involved the extraction of the binder with carbon tetrachloride, removal of the carbon tetrachloride with the aid of a filter stick, and direct titration of the hexahydro-1,3,5-trinitro-s-triazine after addition of dimethylformamide. Of necessity, the sample weight for this procedure was in the order of 0.3 gram. While sharp end points were obtained, the precision of this procedure was far from satisfactory when applied to plant samples. Synthetic samples, on the other hand, gave good precision. It soon became apparent that the hexahydro-1,3,5-trinitro-s-triazine content of individual 0.3-gram samples varied by as much as 5%. This error, probably due to nonuniformity of the sample was overcome by increasing the sample weight to 1.5 grams, and aliquoting. Using this procedure the results obtained on plant samples of composition C-4 gave good precision.

RESULTS

The nonaqueous titration method has been applied to samples of purified hexahydro-1,3,5-trinitro-s-triazine containing no octahydro-1,3,5,7-tetranitro-s-tetrazine (HMX) and synthetic samples of compositions A-3 and C-4 (Tables I, II, and III). In view of the average standard deviation of 0.13% and the average error of 0.13% obtained, as well as the simplicity and rapidity of the developed method, it is recommended that the method be incorporated in applicable government and industrial specifications for the determination of the purity of hexahydro-1,3,5-trinitro-s-triazine and its content in explosive compositions.

ACKNOWLEDGMENT

The author wishes to express his appreciation to E. F. Reese, A. J. Clear, C. J. Bain, Robert Frye, and J. D. Armitage of Picatinny Arsenal for help rendered in the preparation and publication of this report. Appreciation is further expressed to the Ordnance Corps for permission to publish this paper.

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RECEIVED May 10, 1954. Accepted September 30, 1954.

Estimation of Metallic Mercury on the Surface of Tinned Copper

GEORGE T. KERR, SYLVESTER S. MACUT[1], and CARL C. NEELY[2]

Aircraft-Marine Products Inc., Harrisburg, Pa.

An evaporation method has been developed whereby 0.3% of mercury can be estimated on tinned-copper samples weighing approximately 0.7 gram. Accuracy was ascertained by using specially prepared samples containing known quantities of mercury. For samples containing in the order of 2 mg. of mercury, the average difference between the known and estimated content was ±0.21 mg., or an error of approximately ±10%. Application to quality control is promising.

TINNED-copper bodies are produced in quantity for use as various types of contacts in electrical industries. It has been found that "tumbling" these bodies with mercury forms a tin amalgam giving a much improved electrical contact. As an excess of mercury results in the eventual amalgamation and breakdown of copper, it is important that the quantity of mercury deposited on each piece be controlled. Approximately 0.3 to 0.5% of mercury per sample, depending upon the shape of the body, gives excellent results electrically. However, each sample weighs approximately 0.7 gram. Thus, determining the quantity of mercury per sample for a large number of samples utilizing conventional methods would have been tedious.

The amalgamation method (*1*, *2*) for determining mercury was considered. This consists in chemically reducing mercury compounds to metallic mercury, vaporizing the metal, and condensing the vapor on a cooled, tared strip of silver. The mercury is thus weighed directly. In the present problem, chemical reduction would not be necessary, as the mercury is present as the metal. This method was not promising for a large number of analyses, but it led to the development of a similar procedure.

EXPERIMENTAL

One hundred tin-plated copper samples, each weighing approximately 0.7 grams, were numbered with a metal punch for identification, and were individually weighed to the nearest 0.1 mg. using a conventional analytical balance. The weighings were made by a technician with no previous weighing experience. The pieces were placed in a 250-ml. glass-stoppered Erlenmeyer flask and "tumbled" for 1 hour. Six samples were chosen at random and reweighed. The average weight loss was less than 0.1 mg.; the maximum weight loss amounted to 0.2 mg.

To the 100 samples was added 0.2000 gram of mercury. The samples were tumbled with the mercury for 1 hour and again weighed to the nearest 0.1 mg. The data showing weight of mercury deposited on each sample are listed in Table I. The total weight of mercury detected amounted to 0.1863 gram as compared with 0.2000 gram, the amount actually used. Thus the average error is −0.14 mg. per sample, probably attributable to weighing errors.

Apparatus. A 250-ml. round-bottomed flask is fitted to any laboratory-type vacuum pump capable of producing 1 mm. of pressure. The pump is protected with an ice trap. It is preferable that the flask be fitted with either spherical or standard-taper glass joints. The flask is heated in an oil bath containing Dow-Corning silicone oil No. 710.

Test Procedure. The 100 specially prepared mercury-coated samples were placed in the flask and the system was evacuated to 1 mm. of pressure. Meanwhile, the oil bath was heated on an electric hot plate to 190° C. This temperature should be sufficient to effect complete mercury removal at 1 mm., since the vapor pressure of mercury at 190° is approximately 12 mm. The flask and contents were immersed in the bath to a depth

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[1] Present address, Medical School, Temple University, Philadelphia, Pa.

[2] Present address, College of Chemistry and Physics, The Pennsylvania State University, State College, Pa.

Table I. Weights of Mercury Deposited on and Evaporated from Samples

Sample No.	Hg, Mg.		Difference	Sample No.	Hg, Mg.		Difference	Sample No.	Hg, Mg.		Difference	Sample No.	Hg, Mg.		Difference
	Deposited	Evap.			Deposited	Evap.			Deposited	Evap.			Deposited	Evap.	
1	1.7	2.0	−0.3	26	1.5	1.5	0	51	1.7	2.0	−0.3	76	1.5	1.7	−0.2
2	2.0	2.1	−0.1	27	1.0	1.7	−0.7	52	1.7	2.3	−0.6	77	2.2	2.2	0
3	1.9	2.0	−0.1	28	2.0	2.3	−0.3	53	2.0	2.0	0	78	2.2	2.2	0
4	2.1	2.1	0	29	1.5	1.5	0	54	1.3	2.0	−0.7	79	1.8	2.3	−0.5
5	1.9	1.8	0.1	30	1.7	1.7	0	55	1.7	1.7	0	80	1.5	1.5	0
6	2.0	2.0	0	31	2.0	2.0	0	56	2.0	2.2	−0.2	81	2.0	2.1	−0.1
7	2.0	2.0	0	32	2.1	2.5	−0.4	57	1.5	2.4	−0.9	82	2.2	2.5	−0.3
8	2.5	2.3	0.2	33	2.7	2.2	0.5	58	1.5	2.3	−0.8	83	1.4	1.7	−0.3
9	1.8	1.8	0	34	1.7	1.4	0.3	59	2.0	2.2	−0.2	84	1.0	1.6	−0.6
10	2.1	2.1	0	35	1.6	1.7	−0.1	60	1.6	1.7	−0.1	85	2.0	2.0	0
11	2.0	2.1	−0.1	36	1.2	1.2	0	61	1.0	1.5	−0.5	86	1.7	1.7	0
12	2.0	2.0	0	37	1.3	2.1	−0.8	62	2.0	2.0	0	87	2.1	2.1	0
13	1.8	2.0	−0.2	38	2.5	3.0	−0.5	63	2.0	2.1	−0.1	88	1.5	2.0	−0.5
14	1.7	2.1	−0.4	39	2.4	2.8	−0.4	64	2.4	2.2	0.2	89	1.9	2.0	−0.1
15	1.7	1.8	−0.1	40	1.6	1.8	−0.2	65	2.3	2.0	0.3	90	2.6	2.6	0
16	1.4	1.9	−0.5	41	2.0	2.0	0	66	2.0	2.2	−0.2	91	2.0	2.6	−0.6
17	1.8	1.8	0	42	1.8	1.9	−0.1	67	2.3	1.8	0.5	92	1.0	0.8	0.2
18	2.0	1.9	0.1	43	1.8	1.5	0.3	68	1.9	1.9	0	93	2.0	1.9	0.1
19	2.1	2.1	0	44	2.1	2.3	−0.2	69	1.5	1.6	−0.1	94	1.4	2.2	−0.8
20	2.4	2.9	−0.5	45	2.0	1.9	0.1	70	2.1	1.7	0.4	95	2.3	2.3	0
21	2.6	2.6	0	46	1.2	1.2	0	71	2.0	2.0	0	96	2.2	2.2	0
22	1.7	2.0	−0.3	47	1.6	2.2	−0.6	72	1.2	1.4	−0.2	97	1.9	1.9	0
23	1.8	1.8	0	48	1.3	1.3	0	73	1.9	1.9	0	98	1.7	1.7	0
24	2.6	2.4	0.2	59	2.2	2.1	0.1	74	1.4	1.8	−0.4	99	2.3	2.3	0
25	3.2	3.0	0.2	50	1.3	1.9	−0.6	75	2.3	2.5	−0.2	100	1.7	1.8	−0.1
												Total	186.3	199.6	±20.9

such that the liquid level was above the spherical portion of the flask. Every 5 or 10 minutes the flask was gently agitated. After being immersed in the oil bath for 30 to 35 minutes, the flask was allowed to cool in air to room temperature while the vacuum was maintained. The samples were then individually reweighed to the nearest 0.1 mg., and the weight of mercury lost from each sample was then determined by difference. These data are presented in Table I.

That the weight loss suffered on heating was due predominantly to the evaporation of mercury was indicated by the following experiment:

Fifteen tinned-copper samples were numbered, weighed, and subjected to the above test procedure, including agitation. The maximum change in weight amounted to 0.2 mg., the average being 0.11 mg.

RESULTS AND DISCUSSION

The data in Table I indicate that the average difference between the weight of mercury deposited per piece (about 2 mg.) and the weight of mercury estimated by this method is ± 0.21 mg. This amounts to an error of approximately 10%. The probable error is ± 0.19 mg. as computed by standard statistical procedures.

The total weight of mercury added, as determined by the sum of the weights of mercury removed, was 0.1996 gram. This checks remarkably the actual weight of mercury used for tumbling. However, the total weight of mercury added, as determined by the sum of the weights of mercury deposited, amounts to 0.1863 gram, indicating an appreciable weighing error during preparation of samples.

Since preparing the samples containing known weights of mercury necessitates an additional weighing compared with the test procedure for unknown samples, the data reported here probably represent an abnormally high error. Larger quantities of mercury could no doubt be estimated more accurately.

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RECEIVED for review March 5, 1954. Accepted September 29, 1954.

Colorimetric Determination of Trace Quantities of Boric Acid in Biological Materials

W. C. SMITH, JR., A. J. GOUDIE, and J. N. SIVERTSON
Johnson & Johnson Research Center, New Brunswick, N. J.

In a rapid and accurate method for the determination of trace quantities of boric acid in small amounts of biological materials such as blood, urine, and animal tissue, organic matter is destroyed by fusion of the sample with lithium carbonate. The fusion mixture is dissolved in hydrochloric acid; then sulfuric acid is added, followed by a solution of carminic acid. The color develops within 5 minutes. Inorganic materials that are normally found in blood, urine, and animal tissue do not appear to interfere appreciably. Using the Beckman DU spectrophotometer, the method is capable of determining from 2 to 15 γ of boron with an accuracy within ±1.0γ.

IN THE course of evaluating physiological effects of boric acid, it was necessary to determine the boric acid content in small samples of biological material (blood, urine, and animal tissue) in concentrations ranging upward from 1.0 p.p.m. (calculated as boron). Previous workers have developed a variety of methods by which boron has been determined in soils, metals, and various organic materials. Most of these procedures were based on one of the following techniques: identical pH titrations, fluorescence measurements, spectrographic methods, and colorimetric procedures.

When the identical pH electrometric procedure of Wilcox (*15*) was attempted in this laboratory, erratic results were obtained as reported by Berger and Truog (*2*), since the total quantity of boron available for each analysis was usually less than 10 γ.

It was necessary to work with such small samples (2 grams or less) because only limited quantities of material were available from the physiological study referred to above.

White (*14*) and his coworkers recommended determining boric acid by allowing it to react with benzoin and measuring the fluorescence produced by irradiating the complex with ultraviolet light. Although this method is capable of yielding good results under ideal conditions, its accuracy is affected adversely by many of the inorganic ions normally found in biological materials. Furthermore, at the low concentrations of boron present in the biological materials, it would be necessary to use fairly large samples so that the amounts of boron available for analysis would satisfy the sensitivity requirements of the method. To use this method, therefore, it would be necessary to carry out lengthy separations and concentration procedures and to use larger samples than are generally convenient with biological materials; hence, the search was continued for a more rapid and sensitive method.

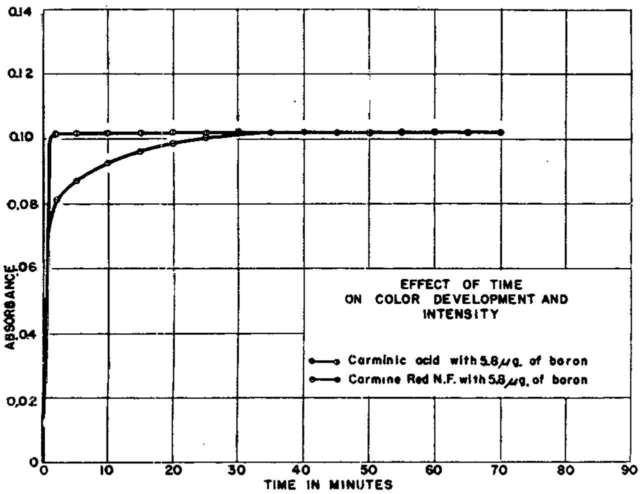

Figure 1

An attempt was made to determine the boron spectrographically, as recommended by Smith, Schrenk, and King (*9*), Melvin and O'Connor (*8*), and Steadman (*10*). This technique was extensively investigated, but, as reported by Melvin and O'Connor, the results were erratic. For example, in a typical series of 15 analyses where the boron concentration in human blood was varied between 1.0 and 10.8 p.p.m., the following relationship was found between the concentration of boron added and recovered (regression line of concentration recovered, *R*, on concentration added, *A*): $R = 1.42A - 0.80$. The standard error of estimate (*16*) of the concentration recovered is 4.01 p.p.m. Therefore, to include about 95% of the points, twice the standard error of estimate must be used. Thus, *R* could be expected to fall within the limits ±8.02 p.p.m. 19 times out of 20. As this error was so large, it was felt that too much additional work would be required to improve the precision of the spectrographic method.

More promising results were obtained by investigating the numerous colorimetric methods reported in the literature (*1, 3–6, 11*). Of these, the quinalizarin procedure (*1*) appeared to be reported most frequently. Although this method is capable of giving good results by visual comparison of the colors under certain conditions, it is of limited value when the color intensities are measured in a spectrophotometer. Weinberg (*12*) and Ellis (*4*) account for this situation by noting that the differences between the absorption spectra of quinalizarin and the boron-quinalizarin complex are very small at nearly all wave lengths. In other words, because of the overlapping spectra of the reagent and the complex, the sensitivity at a given wave length is poor. The eye, on the other hand, is able to integrate all the small differences over the entire visible spectrum and can judge small color and, therefore, small concentration differences.

The method reported by Hatcher and Wilcox (*6*), using carmine No. 40 N.F. as the colorimetric reagent for boric acid, seemed to satisfy best the requirements for a method which is rapid, accurate, relatively unaffected by foreign ions, and reproducible over the desired range of boron concentrations.

The original method of Hatcher and Wilcox was proposed for use on plant materials and natural water, but in adapting it for use in this study the recommended procedure for destroying organic matter—viz., dry ashing with calcium oxide—was too time-consuming when used for biological fluids and tissue.

Wet ashing with concentrated sulfuric acid and hydrogen peroxide has been reported by Hoffman and Lundell (*7*) to give low results owing to volatilization of boron. This was confirmed experimentally by the writers.

Two other methods for destroying organic matter were investigated. These were: dry ashing with a mixture of sodium and potassium carbonates, and dry ashing with lithium carbonate at 650° C. The latter was the most convenient of the methods studied, and was used in the procedure described below.

After the fusion of the biological material with lithium carbonate, the melt was dissolved in hydrochloric acid. A faint yellowish turbidity which developed interfered with the measurement of color intensity. To eliminate the difficulty, the solution obtained by dissolving the melt was centrifuged, and a standard volume of the clear supernatant liquor was used for analysis. No boron could be detected in the precipitate separated by this treatment.

Another critical point in the procedure was found to be the acid concentration required for color development. Satisfactory results were obtained, provided the concentration of sulfuric acid exceeded 90%. Even after the development of the color, precautions were necessary to prevent dilution of the sample by atmospheric moisture.

As reported by Hatcher and Wilcox (*6*), both carmine No. 40 N.F. and carminic acid were satisfactory over most of the range of concentrations investigated. In the present study, the color developed much more rapidly when carminic acid was used. The maximum intensity was reached in tubes stored at room temperature (24° to 27° C.) within 5 minutes after the addition of the reagent, and the color was stable for at least 70 minutes. On the other hand, when carmine No. 40 N.F. was used, the time required to reach the optimum color was 35 minutes (see curves, Figure 1).

METHOD

Apparatus and Reagents. The Beckman DU Spectrophotometer was used with Corex cells of a 1-cm. light path for the absorption measurements. Corning alkali-resistant (low-boron) glass bottles and apparatus, platinum crucibles, and polyethylene sample containers were used wherever possible to minimize chances of boron contamination.

Sulfuric Acid, concentrated, analytical reagent grade. J. T. Baker's reagent grade was found to be substantially boron-free and is recommended.

Carminic Acid Reagent was prepared by dissolving 250 mg. of carminic acid C.P. (Fisher Scientific Co. No. A-93) in concentrated sulfuric acid and diluting to 1 liter with concentrated sulfuric acid.

Aqueous 6*N* Hydrochloric Acid.

Lithium Carbonate, anhydrous, analytical reagent grade. J. T. Baker's reagent grade was found to be substantially boron-free and is recommended.

Preparation of Standard Solutions. Boron stock solution was prepared by dissolving 0.2203 gram of National Bureau of Standard sodium tetraborate decahydrate in distilled water and diluting to 250 ml. The boron content of the concentrated solution was checked using the A.O.A.C. identical pH titration procedure (1 ml. is equivalent to 100 γ of boron). Suitable diluted solutions may be made from this solution by diluting measured portions with distilled water.

Table I. Calibration Summary

Material	No. of Determinations	Calibration Curve	Accuracy Limits[a]
Human blood	19	$B = 56.0A$	±1.0
Human urine	24		
Rabbit blood	19		
Animal tissue	24	$B = 55.0A - 0.5$	±0.8

B. Micrograms of boric acid (as boron).
A. Absorbance reading at 575 mμ.

[a] Accuracy limits (2 × standard error of estimate) will include about 95% of the results.

PROCEDURE

A 0.10-gram sample of lithium carbonate is weighed into a clean platinum crucible. Two milliliters of the fluid (or an appropriate weight of tissue sample) containing not more than 30 γ of boron are transferred into the crucible and, if necessary, excess water is removed by evaporation on the steam bath. The crucible is placed in a muffle furnace and heated for 15 minutes at 100° C., after which the temperature is raised gradually to 650° C., and the heating is continued for 1.5 hours or until the sample is free from carbon. The sample is removed from the muffle furnace and allowed to cool to room temperature, and 2.00 ml. of 6N hydrochloric acid are pipetted into the crucible. The liquid is mixed well by swirling and poured into a 15-ml. centrifuge tube, and the contents are centrifuged at 3000 r.p.m. for 2 minutes or until the supernatant liquor is clear.

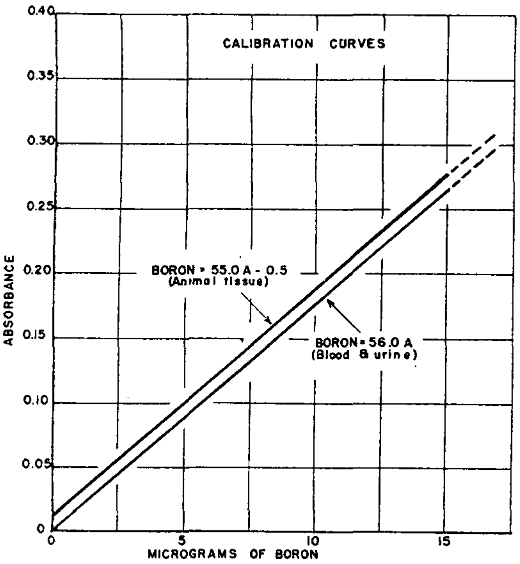

Figure 2

One milliliter of the liquid is transferred into a 100 × 20 mm. alkali-resistant glass test tube (boron-free), 5.00 ml. of concentrated sulfuric acid are added, followed by 5.00 ml. of the carminic acid reagent, and the tube is covered to exclude atmospheric moisture. (Type M. Parafilm was found to be excellent for this purpose. It may be purchased from Para Laboratory Supply Co., Trenton, N. J.) The tube is shaken well, and allowed to stand for at least 5 minutes at room temperature to develop the color, and the absorbance of the solution is measured with a Beckman DU spectrophotometer in a 1-cm. covered cell at 575 mμ.

A blank sample of similar material from which the boron has been removed, if necessary, by the Chapin (*13*) distillation, should be carried through the procedure with each set of samples.

The boron content of the solution is determined by reference to a standard curve made by carrying the biological material containing known quantities of boron through the above procedure.

STANDARDIZATION OF METHOD

The method was standardized by adding known amounts of standard boron solution to 2.0-ml. (or 2.0-gram) boron-free samples of blood, urine, and tissue, which in turn were carried through the procedure. The writers had no difficulty in finding human blood which was boron-free. The blood was checked by ashing a suitable portion in the presence of lithium carbonate and examining the ash with a large quartz spectrograph. No boron lines could be detected on the plates under these conditions, but when 1 p.p.m. of boron was added to the same blood, and the resulting mixture checked in the same manner, the boron lines were easily detected. Urine, on the other hand, frequently contained trace amounts of boron, which were removed as the volatile methyl borate according to the procedure described by Chapin (*13*). The residual material, when made up to its original volume, was then used for the preparation of standards. Animal tissue samples used for calibration studies were also treated by the Chapin technique, when necessary, to remove boric acid.

The absorbance readings at 575 mμ were recorded for the corresponding amounts of boron used and, using prescribed statistical techniques, a calibration curve of boron content on absorbance was computed. There was no significant difference between the calibration curves obtained for various samples of human and rabbit blood and urine. The calibration curve for a variety of animal tissues, however, differed slightly but significantly from the blood and urine curve (Figure 2). These calibration curves are given in Table I along with the accuracy limits [twice the standard error of estimate (*16*)] and the number of determinations performed for obtaining the data.

DISCUSSION

Previous workers (*6*) have reported that nitrates and nitrites were the ions which caused the most serious interferences, but the effects of these are minimized in the procedure by the addition of hydrochloric acid. No other inorganic substances commonly found in biological materials seemed to interfere significantly with the method.

If the boron content of the sample exceeds 30 γ, the proper aliquot should be taken to keep the boron content within the range of the method.

It was found that the following materials could be analyzed satisfactorily by this procedure: blood, urine, lung, heart, thymus, liver, spleen, stomach, duodenum, kidney, prostate or uterus, brain, voluntary muscle, skin, urinary bladder, jejunum, cecum, and terminal colon, as well as fecal matter and gastric contents.

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RECEIVED for review June 22, 1954. Accepted September 17, 1954.

Simplified Procedure for Computing the Growth of Radioactive Decay Products

H. W. KIRBY and D. A. KREMER
Mound Laboratory, Monsanto Chemical Co., Miamisburg, Ohio

A method is described for easing the burden of computing equations for the growth of radioactive decay products. A sample table and a sample calculation are included.

CALCULATION of the growth of decay products from radioactive materials is a fairly simple matter when the decay chain is limited to two or three members. Equations for such calculations were originally proposed by Rutherford (*7*), and can be found in textbooks of radiochemistry (*2*, *4*).

When the decay chain is long, however, as in the radium, thorium, actinium, and neptunium series, the symmetrical expression derived by Bateman (*1*) is customarily used. The general form of a Bateman equation is

$$\frac{N_n(t)}{N_1(0)} = \sum_{i=1}^{n} C_i e^{-\lambda_i t} \qquad (1)$$

where $N_n(t)$ is the number of atoms of the nth member of the decay chain at time t, $N_1(0)$ is the number of atoms of the parent at $t = 0$, and λ_i, the decay constant, is numerically equal to ln 2 divided by the half life, T_i, of the ith member of the chain. C_i is a constant having the form

$$C_i = \frac{\lambda_i \prod_{j=1}^{n-1} \lambda_j}{\prod_{j=1}^{n} \left(\lambda_j - \lambda_i\right)};\ (j \neq i;\ i = 2, 3, \ldots, n-1)$$

$$C_1 = \left(\frac{\lambda_1}{\lambda_n - \lambda_1}\right) \prod_{j=2}^{n-1} \left(\frac{\lambda_j}{\lambda_j - \lambda_1}\right) \qquad (2)$$

$$C_n = \prod_{j=}^{n-1} \left(\frac{\lambda_j}{\lambda_j - \lambda_n}\right)$$

When n is large, evaluation of C_i may become tedious. Mechanical errors which are difficult to detect are not uncommon because of the number of operations involved. Flanagan and Senftle (*3*) have made available tables from which the numerator and denominator of C_i can be evaluated. Use of these tables affords a substantial saving in the time and number of operations required.

However, the authors have found that, by algebraic manipulation, Bateman coefficients can be expressed in a modified form, evaluation of which can, in some cases, be carried out by inspection.

Equations 2 can be transformed as follows:

$$C_i = \left(\frac{T_n}{T_1}\right)\left(\frac{-T_1}{T_1 - T_i}\right)\left(\frac{T_i}{T_i - T_n}\right) \prod_{j=2}^{n-1} \left(\frac{T_i}{T_i - T_j}\right);$$
$$(j \neq i,\ i = 2, 3, \ldots, n-1)$$

$$C_1 = \left(\frac{T_n}{T_1}\right)\left(\frac{T_1}{T_1 - T_n}\right) \prod_{j=2}^{n-1} \left(\frac{T_1}{T_1 - T_j}\right)$$

$$C_n = \left(\frac{T_n}{T_1}\right)\left(\frac{-T_1}{T_1 - T_n}\right) \prod_{j=2}^{n-1} \left(\frac{T_n}{T_n - T_j}\right) \qquad (3)$$

The function $\frac{T_i}{T_i - T_j}$ has the interesting property that, in practice, it is usually either slightly greater than unity or slightly less than zero. This property makes it possible to evaluate many Bateman coefficients by inspection, for if any C_i contains at least one $\frac{T_i}{T_i - T_j}$ which is approximately equal to zero, then that C_i may be taken as equal to zero. In such cases, $C_i e^{-\lambda_i t}$ converges rapidly to zero and can be neglected for most practical purposes.

In the special case where the nth decay product is stable, a slightly different procedure is followed, since Equation 3 is not valid when one of the half lives is infinite. In this case, T_n is treated as a very large finite number, and Equations 3 are rearranged as follows:

$$C_i = \left(\frac{T_i}{T_1}\right)\left(\frac{T_1}{T_1 - T_i}\right) \prod_{j=2}^{n-1} \left(\frac{T_i}{T_i - T_j}\right);$$
$$(i = 2, 3, \ldots, n-1) \qquad (4)$$

$$C_1 = -\prod_{j=2}^{n-1} \left(\frac{T_1}{T_1 - T_j}\right);\ C_n = 1$$

The present communication is abstracted from a report (*6*) which includes tables of the function $\frac{T_i}{T_i - T_j}$ for every possible combination of two radionuclides in the four decay series. Table I is a portion of one of these tables. The nuclear data were taken from the table of isotopes compiled by Hollander et al. (*5*). In each case, the preferred (first listed) half-life value was used.

The following sample calculation illustrates the use of the tables: Required: the equation of growth of radon-220 from thorium-232.

$$\frac{N_6(t)}{N_1(0)} = C_1 e^{-\lambda_1 t} + C_2 e^{-\lambda_2 t} + \ldots + C_6 e^{-\lambda_6 t}$$

$$= \frac{T_6}{T_1}\left(k_1 e^{-\lambda_1 t} + k_2 e^{-\lambda_2 t} + \ldots + k_6 e^{-\lambda_6 t}\right)$$

$$\frac{T_6}{T_1} = 1.24247417 \times 10^{-16}$$

k_1 = 1.00000000 (by inspection of column under Th^{232})
k_2 = (−1) (1.00010439) (1.39583333) (1.00148968) × (1.00000026)
= −1.39805897 (from column under Ra^{228})

Table I. $\frac{T_A}{T_A - T_B}$; **Thorium Series (4*n*)**

		A					
Half Lives	*B*	Th^{232}	Ra^{228}	Ac^{228}	Th^{228}	Ra^{224}	Rn^{220}
1.39 × 10^{10} years	Th^{232}	. . .	0.00000000	0.00000000	0.00000000	0.00000000	0.00000000
6.7 years	Ra^{228}	1.00000000	. . .	−0.00010439	−0.39583333	−0.00148968	−0.00000026
6.13 hours	Ac^{228}	1.00000000	1.00010439	. . .	1.00036819	1.07546473	−0.00247575
1.90 years	Th^{228}	1.00000000	1.39583333	−0.00036819	. . .	−0.00527292	−0.00000091
3.64 days	Ra^{224}	1.00000000	1.00148968	−0.07546473	1.00527292	. . .	−0.00017332
54.5 seconds	Rn^{220}	1.00000000	1.00000026	1.00247575	1.00000091	1.00017332	. . .

$$
\begin{aligned}
k_3 &= (-1)(-0.00010439)(-0.00036819)(-0.07546473)(1.00247575) \\
&= 0.00000000 \text{ (from column under Ac}^{228}\text{)} \\
&\quad \text{or, by inspection, } (1 \times 10^{-4})(4 \times 10^{-4})(8 \times 10^{-2}) = 3.2 \times 10^{-9} \cong 0.00000000 \\
k_4 &= (-1)(-0.39583333)(1.00036819)(1.00527292)(1.00000091) \\
&= 0.39806740 \text{ (from column under Th}^{228}\text{)} \\
k_5 &= (-1)(-0.00148968)(1.07546473)(-0.00527292)(1.00017332) \\
&= -0.00000845 \text{ (from column under Ra}^{224}\text{)} \\
k_6 &= (-1)(-3 \times 10^{-7})(-2 \times 10^{-6})(-9 \times 10^{-7})(-2 \times 10^{-4}) \\
&\cong 0.00000000 \text{ (from column under Rn}^{220}\text{)}
\end{aligned}
$$

Therefore,

$$
\frac{N_6(t)}{N_1(0)} = 1.24247417 \times 10^{-16} (e^{-\lambda_1 t} - 1.39805897\, e^{-\lambda_2 t} + 0.39806740\, e^{-\lambda_4 t} - 0.00000845\, e^{-\lambda_5 t}
$$

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RECEIVED for review June 14, 1954. Accepted October 14, 1954. Mound Laboratory is operated by Monsanto Chemical Co. for the United States Atomic Energy Commission under Contract Number AT-33-1-GEN-53.

The Rank Correlation Method

JOHN T. LITCHFIELD, JR., and FRANK WILCOXON

Stamford Research Laboratories and Lederle Laboratories Division, American Cyanamid Co., Stamford, Conn., and Pearl River, N. Y.

The usefulness of the rank correlation method has been increased by providing a table of critical totals for two levels of probability and a nomograph which gives the rank correlation coefficient directly. Use of the method is illustrated.

CORRELATION or association between two variables is widely used as a basis for product control and evaluation. Frequently it is difficult, time-consuming and, hence, costly to measure directly that property of a product which is of major interest. In such cases, search is made for some other property which can be measured quickly, easily, inexpensively, and which is closely related to the property of major interest. Generally, a number of properties are examined and that one showing the best correlation is selected.

A rapid test for correlation is very useful, as it circumvents unnecessary computations of correlation coefficients in cases where there is poor or insignificant correlation. The Spearman rank correlation method (*4, 6, 9*) is generally satisfactory for this purpose, and like other quick methods for detecting association (*4, 7*) is nonparametric—i.e., the normality of the distribution sampled need not be assumed. However, the tables of critical totals given by Olds (*6*), Kendall (*4*), and Dixon and Massey (*1*) are brief or incomplete. Furthermore, the values given by Dixon and Massey are for a probability of 0.05 and 0.01, only if the experimenter is interested in a one-sided test. In many cases one is interested in the probability of exceeding the critical value of the coefficient in a + or − direction, and wishes to work at the 5 or 1% level of significance.

In order to facilitate use of the rank correlation method, a table of critical totals of squared rank differences and a nomograph which permits direct reading of the rank correlation coefficient were prepared covering 6 to 40 pairs of observations and two probability levels, 0.05 and 0.01.

These probability levels are for the case described above in which the test is made for presence of correlation whether positive or negative, and if in this test an observed total falls within the critical limits, the conclusion will be reached that the correlation may well be zero—i.e., there is no correlation.

COMPUTATIONS

The Spearman rank correlation coefficient, designated as ρ, has limits of +1 to −1, and is obtained by solution of:

$$
\rho = 1 - \left[\frac{6\Sigma(\text{R.D.})^2}{n(n^2 - 1)}\right] \tag{1}
$$

where R.D. = difference between paired ranks, n = number of pairs, and Σ = summation over n values of $(\text{R.D.})^2$.

Kendall (*4*) calculated the exact distribution of the sum of squared rank differences for values of n from 1 through 8 and found considerable departure from normality, particularly in the tails of the curve. He showed that for values of n greater than 8 and less than 21, a good approximation was given by making use of Student's t distribution as:

$$
t = \rho \sqrt{\frac{n-2}{1-\rho^2}} \tag{2}
$$

Substituting the expression for ρ (Equation 1) and solving for $\Sigma(\text{R.D.})^2$ gives:

$$
\Sigma(\text{R.D.})^2 = \frac{n^3 - n}{6}\left[1 \pm \sqrt{\frac{t^2}{n - 2 + t^2}}\right] \tag{3}
$$

Using the values of t for $p = 0.05$ or 0.01, and $n-2$ degrees of freedom, this equation was solved for the lower and upper critical totals of squared rank differences for all values of n from 8 through 40. Values of t were taken from the table in Snedecor (*8*), except in the case of t for 31 through 34 and 36 through 38 degrees of freedom, where graphic interpolation was used. The accuracy of the interpolated figures was equal to that of the tabular values.

In Table I, the lower and upper critical totals of squared rank differences for probabilities of 0.05 and 0.01 are given for $n = 5$ to $n = 40$. The values for n from 5 through 8 were taken from Kendall (*4*), while those for n from 9 through 40 were obtained as described above using Equation 3.

To obtain the rank correlation coefficient, ρ, Equation 1 must be solved. To eliminate this computation a nomograph was prepared. The equation for the nomograph was derived by rearranging Equation 1 to give:

$$(1 - \rho) = \left[\frac{6}{n(n^2 - 1)}\right] [\Sigma(\text{R.D.})^2]$$

The most satisfactory form of nomograph was found to have n and ρ for outer scales and $\Sigma(\text{R.D.})^2$ for the middle scale. The method for construction of the nomograph is described by Levens (*5*) and Douglas and Adams (*2*).

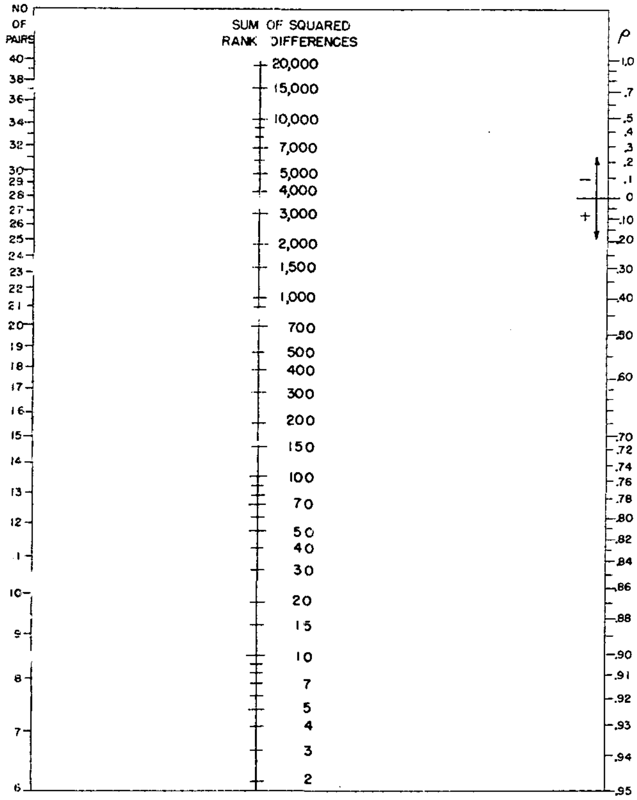

Figure 1. Nomograph for obtaining ρ, the rank correlation coefficient

Table I. Critical Values of $\Sigma(\text{R.D.})^2$ for the Rank Correlation Method[a]

Use of table. If the observed total is equal to or less than the appropriate lower tabular value, or equal to or greater than the appropriate higher tabular value, the correlation is significant for that probability. High values correspond to negative correlations, and low values to positive correlations.

	$\Sigma(\text{R.D.})^{2a}$ Probability			$\Sigma(\text{R.D.})^2$ Probability	
n[b]	0.05	0.01	n	0.05	0.01
..	...	...	21	873–2207	695–2385
..	...	...	22	1022–2520	820–2722
..	...	...	23	1187–2861	960–3088
..	...	...	24	1370–3230	1115–3485
5	0–40	...	25	1570–3630	1287–3913
6	4–66	0–70	26	1789–4061	1475–4375
7	12–100	4–108	27	2028–4524	1681–4871
8	22–146	10–158	28	2287–5021	1906–5402
9	40–200	24–216	29	2569–5551	2149–5971
10	61–269	39–291	30	2873–6117	2414–6576
11	88–352	58–382	31	3199–6721	2700–7220
12	121–451	84–488	32	3550–7362	3008–7904
13	163–565	115–613	33	3926–8042	3338–8630
14	213–697	154–756	34	4328–8762	3693–9397
15	272–848	201–919	35	4757–9523	4073–10,207
16	342–1018	257–1103	36	5213–10,327	4476–11,064
17	423–1209	322–1310	37	5698–11,174	4908–11,964
18	515–1423	398–1540	38	6213–12,065	5366–12,912
19	621–1659	484–1796	39	6758–13,002	5853–13,907
20	740–1920	583–2077	40	7334–13,986	6367–14,953

[a] $\Sigma(\text{R.D.})^2$ = sum of squared rank differences.
[b] n = number of pairs ranked.

USE OF RANK CORRELATION METHOD

The rank correlation method is applied to n pairs of observations on two variables, x and y, as follows:

All the x values are ranked by assigning rank number 1 to the lowest value, 2 to the next higher value, and so on up to n, the highest rank number. In the event of two or more identical values, the average value of the rank numbers involved is assigned. [Kendall (*4*), Woodbury (*10*), and DuBois (*3*) give methods of correcting ρ for cases of tied ranks. However, when ties are not very numerous, this correction makes little difference in the final result. An exact correction for confidence limits when ties occur appears not to have been published.] The y values are then ranked independently from 1 to n (low to high). Next, each y rank is subtracted from its corresponding x rank to give n rank differences, and these should add to zero as a check on the arithmetic. Each rank difference is squared and the sum of the squared rank differences is accumulated, $\Sigma(\text{R.D.})^2$. With $\Sigma(\text{R.D.})^2$ for n pairs, reference to Table I indicates whether the correlation is significantly different from zero at either the 5 or 1% level of significance, while a straight-edge placed on the nomograph gives an estimate of ρ, the rank correlation coefficient.

EXAMPLE

In the production of a special blend of corrosion inhibitors, uniformity from one batch to another was controlled by analysis for per cent zinc. This was an expensive control because the analysis was very slow, and production batches had to be held in storage. A study was made, therefore, to find other methods of analysis which were quick and easy and which were correlated with per cent zinc. The following data were obtained when both per cent zinc and per cent ash were determined on each of eleven different batches. Per cent zinc and ash were ranked separately and $\Sigma(\text{R.D.})^2$ was obtained as shown below:

Batch No.	Zinc, %	Ash, %	Rank of		Rank Difference	$(\text{R.D.})^2$
			Zinc	Ash		
1	5.33	17.97	2.5	5.0	−2.5	6.25
2	5.63	18.13	11.0	9.0	+2.0	4.00
3	5.42	18.06	9.0	8.0	+1.0	1.00
4	5.39	18.05	7.0	7.0	0.0	...
5	5.45	18.16	10.0	10.0	0.0	...
6	5.33	17.75	2.5	1.5	+1.0	1.00
7	5.36	17.75	4.0	1.5	+2.5	6.25
8	5.37	17.92	5.0	3.5	+1.5	2.25
9	5.39	18.01	7.0	6.0	+1.0	1.00
10	5.39	19.02	7.0	11.0	−4.0	16.00
11	5.30	17.92	1.0	3.5	−2.5	6.25
					+9.0	$\Sigma(\text{R.D.})^2$ = 44.00
					−9.0	

Reference to Table I for n = 11 reveals that the observed total 44, is less than the critical total of 58 for p = 0.01.

There is, therefore, a significant positive correlation, and there is less than 1 chance out of 100 that an observed correlation as large as or larger than a correlation corresponding to a critical total of 58 is due to experimental error. The rank correlation coefficient ρ was read from the nomograph, by connecting with a transparent straight-edge n = 11 and $\Sigma(\text{R.D.})^2$ = 44, as ρ = +0.8.

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RECEIVED for review March 26, 1954. Accepted October 14, 1954. Full-size copies of the nomograph will be made available by the authors on request.

Calcium Carbonate as an Internal Standard for Quantitative Infrared Analysis

L. E. KUENTZEL

Wyandotte Chemicals Corp., Wyandotte, Mich.

Calcium carbonate is a good internal standard for quantitative analysis of solids by infrared absorption methods. It is stable and inert, has three widely spaced sharp bands, and may be obtained as a pure powder with all particles less than 1 micron in diameter.

CALCIUM carbonate is a useful internal calibration standard for the quantitative analysis of solids by infrared absorption methods. It is stable chemically and physically, is inert to the action of many other solids, and may be obtained in pure form with all particles less than 1 micron in diameter. The latter property eliminates the need for tedious mulling. Moreover, its infrared spectrogram exhibits three widely spaced, relatively sharp and strong absorption bands with transparent regions in between (*2*, *3*).

Actually, the quantitative analysis of mixtures of solid materials by infrared is most conveniently carried out in suitable solvents. If this is not possible, the solid materials must be handled as suspensions in mineral oil or potassium bromide pellets. Where sample or cell thickness cannot be determined accurately, the use of internal standards becomes necessary. While internal standards are often used in quantitative analytical procedures, an inorganic material as such finds rather limited use in the field of infrared.

It was necessary to calibrate for the quantitative analysis of mixtures of sodium toluene sulfonate and alkyl benzene sulfonate by infrared methods. The former is a dry, easily mulled solid, while the latter is waxlike. After a rather thorough search failed to reveal a common solvent which was relatively transparent in the analytical regions of the spectrum, it was decided to use an internal standard and calibrate with mineral oil suspensions. Calcium carbonate proved to be satisfactory, but the alkyl benzene sulfonate could not be mulled into suspension in mineral oil. This difficulty was overcome by dissolving the waxlike component in ethyl ether, mixing this solution with the mineral oil, and then precipitating the alkyl benzene sulfonate into the oil by evaporating the ether.

PROCEDURE

Weigh exactly 1 gram of sample, transfer it to the mortar of a Fisher motor-driven grinder, and add 0.0100 gram of Purecal U (Wyandotte Chemicals Corp.) calcium carbonate.

Wash down the sides of the mortar with about 5 ml. of ethyl ether, so as to collect sample and standard in the bottom. Stir slowly with a spatula until all the alkyl benzene sulfonate has dissolved. The sodium toluene sulfonate and calcium carbonate do not dissolve.

Add about 5 ml. of mineral oil by pouring it around the sides of the mortar and allow it to run into the ether solution. This prevents the evaporation of ether from the mortar walls, with resultant deposition of a film of alkyl benzene sulfonate.

Adjust the mortar wall scraper to sweep the mineral oil and ether toward the center of the mortar as it rotates. Do not lower the pestle into the mixture. Adjust a heat lamp about 12 inches from the mortar, so as to warm the mixture and drive off the ether. The rotation of the turntable and the gentle sweep of the scraper produce a gradual mixing of ether and mineral oil, with the mineral oil always concentrated at the outer edge. Small quantities of mineral oil may be added from time to time to keep the sample washed down into the mortar. The total amount of oil used should be about 10 ml.

After the ether has evaporated, the pestle may be lowered into the mortar and normal mulling continued.

The effect of mulling time on the absorbance measurements was studied. This factor was without effect upon the absorbances of the alkyl benzene sulfonate and calcium carbonate, as might be expected from the fact that both exist in extremely small particle size before mulling, but the absorbances of the sodium toluene sulfonate became progressively greater with increased grinding time as a larger and larger proportion of the material was ground to sufficiently fine particle size. After 2 hours of mulling there was no appreciable change. However, the correlation between mulling time and absorbance values obtained from measurements on sodium toluene sulfonate bands was constant, so that reproducible absorbance measurements could be obtained from samples mulled for any arbitrary time of 30 minutes or more.

The calibration from known synthetic mixtures and the analysis of unknown mixtures were carried out by the method used by Barnes *et al.* (*1*).

A single-beam, custom-built infrared spectrograph was used and the familiar cell-in, cell-out method of obtaining the incident and transmitted energy was employed. An 0.03-mm. platinum spacer was used in the infrared cell to obtain approximately reproducible sample thicknesses. Because the mull could not be introduced into the cell in the normal manner, it was necessary to disassemble it each time, place a drop or two of mull on the lower window inside the shim, and reassemble. The ratio of the absorbance measured at the 11.4-micron band of calcium carbonate to that measured at a characteristic band for each of the other two materials was plotted against the concentration of the corresponding compound in the synthetic mixture and two straight-line calibration curves resulted. Interest was in the analysis of approximately 50–50 mixtures of the two sulfonates, so the calibration covered concentrations of 40 to 60% of each in the other.

An average deviation from the mean of slightly less than 1% was obtained from repeated analyses of a given sample and the average deviation from the true value for repeated analyses of the synthetic mixtures was the same.

ACKNOWLEDGMENT

The author wishes to thank the Wyandotte Chemicals Corp., Wyandotte, Mich., for permission to publish this work and to acknowledge the helpful assistance of Dolores Phillips.

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RECEIVED for review May 14, 1954. Accepted October 30, 1954. Presented before the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, March 1 to 5, 1954, Pittsburgh, Pa.

Determination of Specific Surface of Sieve-Size Powders

BERNARD DUBROW and MARY NIERADKA

Pyrotechnics Chemical Research Laboratory, Picatinny Arsenal, Dover, N. J.

Instruments available for measurement of specific surface by air permeability have been generally utilized for materials in the subsieve range. This work extends the range of air permeability measurements to sieve-size powders. An instrument which employs a sensitive adjustable flowmeter was developed. The accuracy and precision of the method indicate that the instrument can readily be adopted for rapid routine measurements of specific surface or average particle size in the range investigated.

THERE are two widely adopted air permeability methods for the measurement of specific surface: a method developed by Gooden and Smith (*4*), and the Blaine method (*1*). These methods are satisfactory when applied to subsieve powders. The sensitivity of these measurements, however, decreases considerably for materials having average surface diameters greater than 50 microns.

A need existed for an air permeability method with a wider range and greater sensitivity, because for many applications of coarse powders the specific surface, rather than the mean diameter determined by microscopic count or by sieve analysis, is of fundamental importance, especially where chemical reactions are involved.

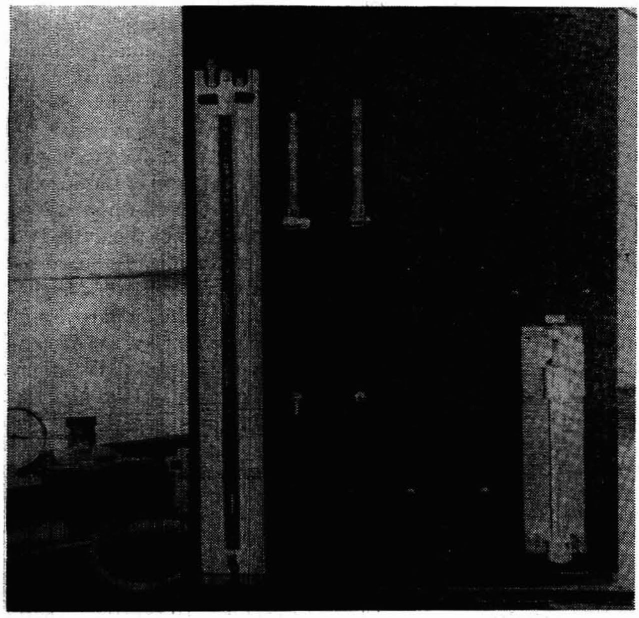

Figure 1. Front view of particle-size apparatus

To meet this need, a method that extends the utility of the air permeability method to powders in the sieve particle-size range was developed. This method is simple, rapid, and readily applicable to routine measurement.

GENERAL AIR PERMEABILITY METHOD

The theory of the permeability method and details of experiments conducted with liquids have been discussed by Carman (*2, 3*). Based on Carman's theory, Lea and Nurse (*5*) developed a rapid air permeability method for measuring specific surface of subsieve powders. The commercially available apparatus developed by Blaine is a simplification of the Lea and Nurse equipment. Another apparatus based on the air permeability principle, which is suitable for rapid routine operation, was developed by Gooden and Smith (*4*). The method described in this paper for measuring the average particle size of materials in the sieve-size range is based on the Gooden and Smith method.

The air permeability measurement of specific surface, which, if desired, can be expressed as average surface diameter, is based on the relation between the rate of flow of air at low pressures through packed beds and the external surface of the packing. In carrying out the measurements, three properties of the packed bed of powder are measured: its physical dimensions, the pressure drop across it, and the rate of flow of air through it. The air permeability apparatus uses a manometer to indicate pressure drop and a flowmeter to measure the rate of flow of air at constant pressure.

For materials having an average surface diameter between 2 and 50 microns, the precision with which differences in manometric readings can be made with existing equipment is higher than the over-all reproducibility of the test. This, however, is not the case for materials in the sieve particle-size range. For these coarser materials the reliability of the flowmeter readings decreases and, consequently, results obtained for these samples are in doubt, owing to the inadequacy of the flowmeter. In order to extend the range of the existing equipment it is necessary to incorporate a more sensitive and adjustable flowmeter. In addition to the flowmeter it appeared desirable, for measuring coarse materials, to increase the sample size and to alter the packing technique.

APPARATUS

Photographs of the apparatus that was developed are shown in Figures 1 and 2. The line diagram, Figure 3, shows the essential experimental parts of the equipment.

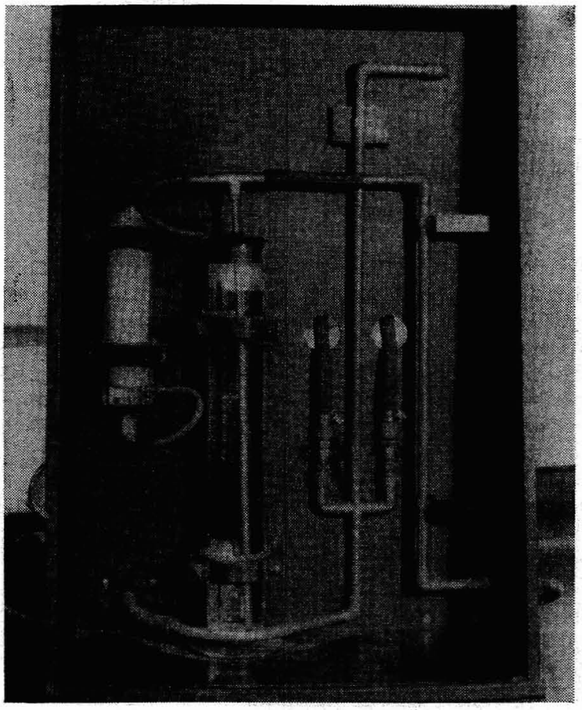

Figure 2. Back view of particle-size apparatus

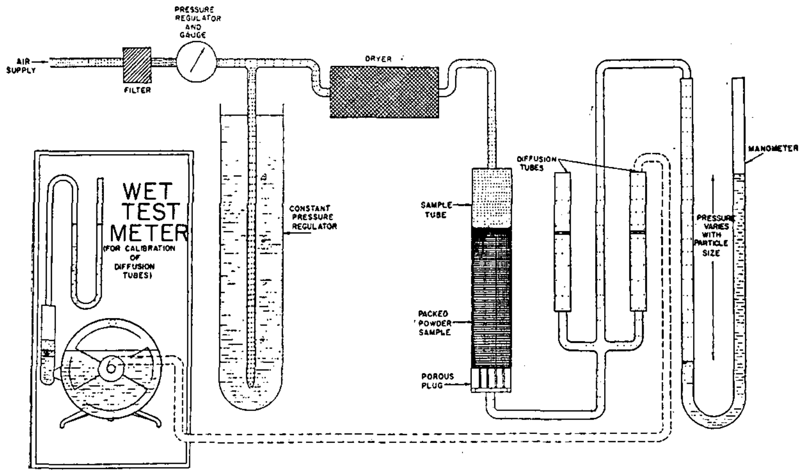

Figure 3. Line diagram of equipment

Air is introduced into the apparatus through a reducing valve at approximately 2-pound gage pressure, then passes through a drying bottle which is connected to both a standpipe and a sample tube. The standpipe, filled with water, serves to fix the pressure of the air at the point of introduction to the sample. The excess pressure is released as bubbles escaping through the water. The air at this constant pressure flows downward through the sample tube in which the powder to be tested is contained. A porous plug supports the powder in the tube. After leaving the sample tube, the air is divided along two paths: through an air resistance tube, and into a water manometer. The air resistance tube is a fritted-glass diffusion tube for which the rate of air passing through it has been determined by means of a wet-test meter. The manometer and the resistance formed by the air resistance tube together constitute a flowmeter. If a sample of powder is added to the sample tube, the difference in pressure as read on the manometer, with and without the sample in the apparatus, will indicate the resistance of the powder sample to a constant air flow. Before the particle size of a sample can be determined, calibration is necessary.

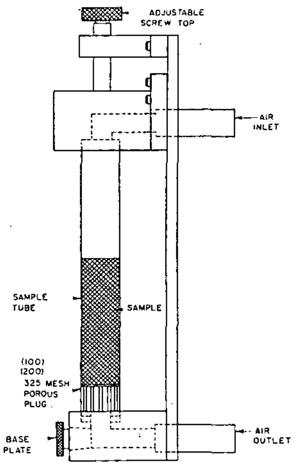

Figure 4. sample tube assembly

CALIBRATION PROCEDURE

Calibration of the apparatus consists of determination of the permeability constants of coarse, medium, and fine glass diffusion tubes used as flowmeter resistances. The calibration procedure is schematically illustrated in the line diagram (Figure 3). The wet-test meter is connected to the bottom end of the air resistance tube. With the empty sample tube inserted in the air permeability apparatus, air is passed through the system. When air is observed to pass through the wet-test meter at a constant rate, the initial volume reading, V_1, and the time of the reading, t_1, are recorded. The deflection of the water in the manometer indicating the air pressure, P, in the system is read directly. After 1 hour the volume reading and the time of the second reading, V_2 and t_2, respectively, are recorded. The permeability constants, K, of the air resistance tubes are calculated by means of Equation 1:

$$K = 1.263 \times 10^3 \left[\frac{V_2 - V_1}{(t_2 - t_1)P}\right]^{1/2} \qquad (1)$$

TEST PROCEDURE FOR DETERMINING AVERAGE PARTICLE SIZE

To determine the surface diameter of a sample the procedure is as follows:

A weighed sample of dry powder equal in grams to an integral multiple of the true density of the material is poured into the sample tube containing a porous plug. The sample is lightly tapped, and inserted in the apparatus (Figure 4). With the air regulated to permit approximately two or three air bubbles to escape through the standpipe per second, a calibrated glass diffusion tube is selected so that the pressure reading, F, is approximately 0.5 the initial pressure, P. The manometric deflections, F and P, are recorded. The sample tube is removed, and the height of the sample, H, is measured with a modified depth gage.

CALCULATION OF PARTICLE SIZE

The average surface diameter of the powder can be calculated by means of the Gooden and Smith equation (*4*).

$$\mu = \frac{KHN}{(AH - N)^{3/2}}\sqrt{\frac{F}{P - F}}$$

where

μ = surface weighted diameter, microns
K = permeability constant, cm.$^{3/2}$, of air resistance tubes
H = sample height, cm.
A = cross-sectional area, sq. cm., of sample tube
P = initial air pressure, grams per sq. cm.
F = exit air pressure, grams per sq. cm.
N = weight of sample per density

Nomograms, based on the Gooden and Smith equation, have been constructed to simplify the calculations (Figures 5, 6, and

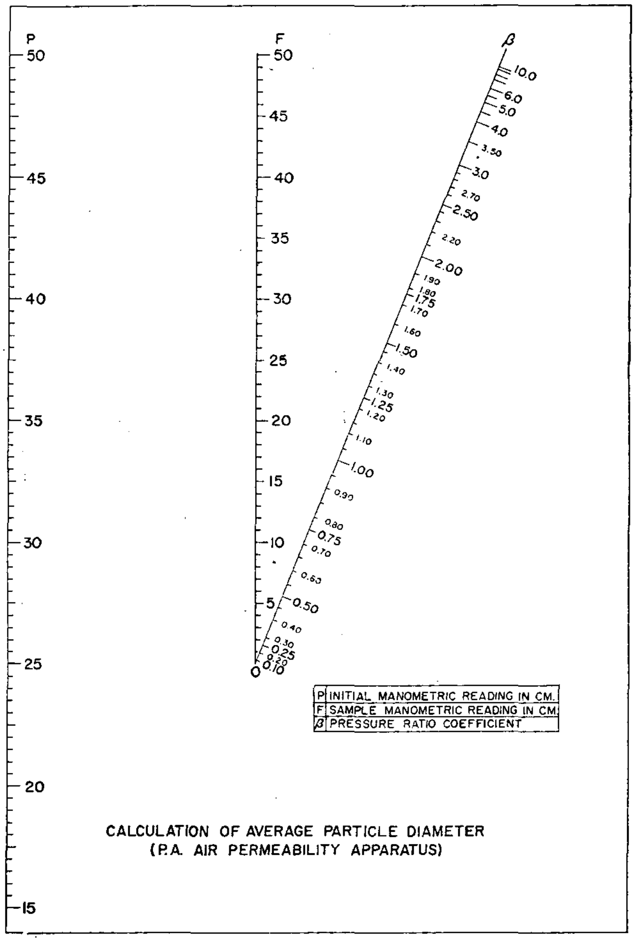

Figure 5. Nomogram for determination of β

Table I. Particle Size of Atomized Magnesium Determined by Three Methods

Magnesium Screened Fractions, Mesh	Screen Analysis, μ	Microscopic, μ	Air Permeability, μ
16/20	1000	990	742
20/30	705	703	620
30/40	498	510	451
40/50	353	350	346
50/70	250	255	233
70/100	177	182	170
100/140	125	118	116
140/200	88	85	79
200/325	57	51	58
Production Samples			
No. 1	360	370	379
No. 2	112	112	101

7). β, equated to $\sqrt{F/P - F}$, is obtained using nomogram I (Figure 5). α, equated to $KHN/(AH - N)^{3/2}$, where N is 10, can be determined by means of nomogram II (Figure 6). α and β, when aligned in nomogram III (Figure 7), give a value in microns corresponding to the average particle size of the material tested.

DISCUSSION

The accuracy and precision of the permeability method depend to a great extent on the preparation of a uniform bed of material. For coarse samples it was found necessary to pour and tap the material in the sample tube. This technique gives uniform packing, which eliminates the possibility of fracturing coarse friable salts. A larger sample of powder than is ordinarily used in permeability measurement of particle size was selected in order to provide a larger total surface and, thereby, increase the sensitivity of the method.

Filter paper disks, which usually are employed in the commercial Blaine and Gooden and Smith methods to contain the sample, were not used, because they were found to offer significant resistance to the air flow, compared with the resistance of the coarse sample. In the present design, plugs covered with wire mesh were used in place of filter paper.

In order to determine the accuracy of the method, a series of comparative tests was made for sieve-size atomized magnesium powder having a mean length diameter ranging from approximately 50 to 1000 microns. Results of the average particle size as determined by screen analysis, microscopic count, and the air permeability method, are given in Table I.

As the magnesium particles are generally spherical, the diameter of the individual particles could be measured microscopically with a filiar micrometer. The geometric mean of 100 particles for each sample is recorded in Table I.

The sieve analysis geometric mean values of the production samples were obtained by plotting on logarithm probability paper the per cent of the sample greater than a particle size corresponding to a sieve opening against the size of the sieve opening. The mean value was obtained by reading the 50% size. The geometric mean of each sieve fraction was obtained by averaging the openings of the upper and lower sieves.

The experimental trend that was established indicates that the surface diameters were generally equal to or lower than the geometric mean length diameters. This is in agreement with the expected theoretical trend.

If the average mean length diameter of the magnesium par-

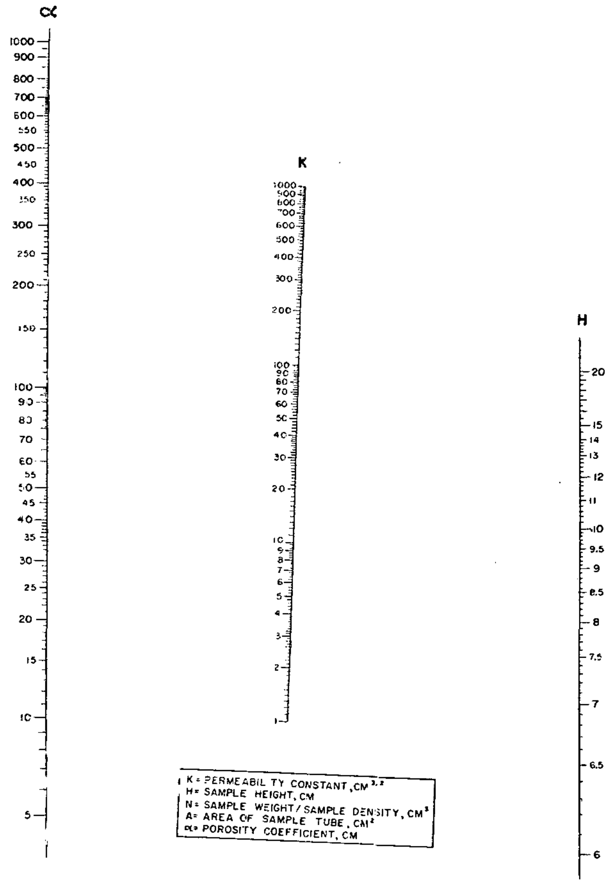

Figure 6. Nomogram for determination of α for $N = 10$

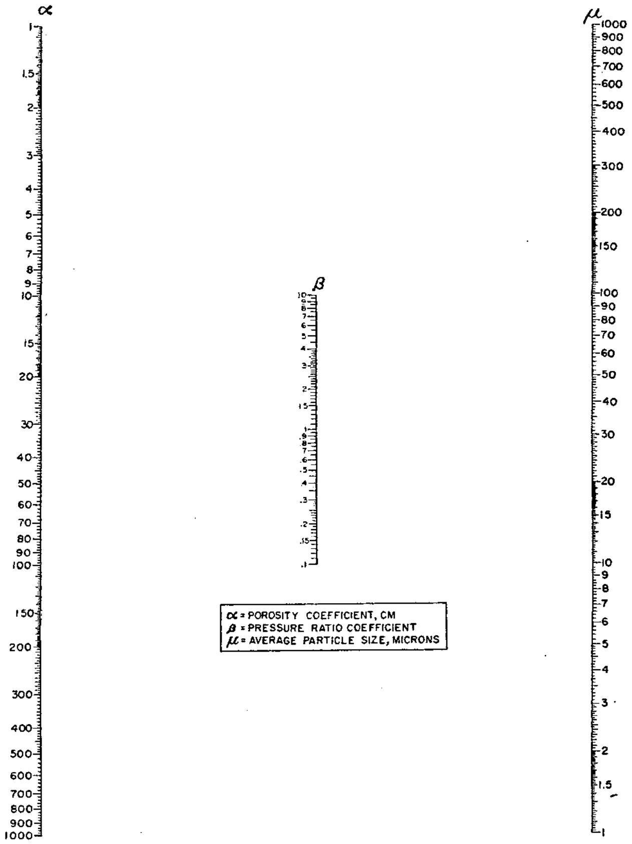

Figure 7. Nomogram for determination of average particle diameter

ticles is considered constant, any deviation of the particles from sphericity would increase the surface per unit weight of material. Thus, the addition of irregular particles to a batch of perfectly spherical particles would increase the average surface of the batch, and consequently decrease the average surface diameter per unit weight. In view of this, the air permeability geometric mean and the mean obtained from sieve analysis and microscopic count should be equal if the particles are perfect spheres. If the particles are not spherical, the air permeability geometric mean should be less than the mean length diameter.

Visual examination of the coarse fractions indicated that the particles had indentations and were not as smooth as the fine fractions. The coarse fractions, as expected, gave low air permeability values. The discrepancy in values of surface and mean length diameters for the coarse fractions is not an indication that the air permeability method is less accurate than the microscopic or sieve analysis methods. It only emphasizes the fact that different physical dimensions are considered. As chemical reactions are more dependent on the surface of powders than on diameters, the air permeability values, which consider the surface of materials, are preferred.

Table II compares the values of average diameter obtained by the three experimental methods for barium nitrate. Since barium nitrate is crystalline, the air permeability values are expected to be lower than the screen analysis and microscopic values.

Nontechnical personnel could be readily trained to determine the average size of a powdered sample. The time required for a single determination is less than 10 minutes.

The average reproducibility of the results is less than 5% over the range of 50 to 1000 microns. Three times the standard deviation was taken as a measure of reproducibility. Below 50 microns the reproducibility decreases.

Table II. Particle Size of Barium Nitrate Determined by Three Methods

Production Samples	Screen Analysis, μ	Microscopic, μ	Air Permeability, μ
No. 1	580	650	495
No. 2	315	340	303

ACKNOWLEDGMENT

The authors are indebted to David Hart for his cooperation and guidance, and to Henry Eppig and Garry Weingarten for their contributions. George Kottler, Howard Keyes, Paul Gallien, George Nichols, and Ray Carlstrom aided in the preparation of the nomograms and diagrams.

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Received for review June 25, 1954. Accepted September 29, 1954. Presented before the Division of Industrial Chemistry at the Meeting-in-Miniature, American Chemical Society, Newark, N. J., 1954.

Separation and Determination of Microgram Quantities of Silver, Mercury, and Copper with Dithizone

HARALD FRIEDEBERG

Chemistry Division, British Columbia Research Council, Vancouver 8, B. C., Canada

Separation and determination of silver, mercury, and copper by dithizone extraction appear to be more rapid than by other procedures, if use is made of the complexing of copper by ethylenediaminetetraacetic acid, the effect of chloride on the extraction of silver at different pH, and the decomposition of the dithizonates of mercury and copper by 6*N* hydrochloric acid. A complete analysis can be carried out in 2 hours.

SILVER, mercury, and copper can be extracted from acid solution (pH 1 to 2) by dithizone in carbon tetrachloride or chloroform (Table I). For their separation and determination the following methods are available:

Silver is usually determined by a mixed-color method using copper dithizonate for extraction, which makes separation of copper unnecessary (*6*). In the presence of mercury, silver cannot be determined directly with dithizone and mercury has to be removed by ignition of the precipitated sulfides (*5*).

Mercury is separated from copper by fractional extraction from 0.075 to 0.1 *N* hydrochloric acid solution (*2*), or by shaking the carbon tetrachloride solution of the dithizonates with acid bromide solution. Copper remains in the carbon tetrachloride, while mercury passes into the aqueous phase and can be re-extracted by dithizone after being made alkaline (*1*). Alternatively, mercury can be transferred to the aqueous phase by shaking the carbon tetrachloride solution of the dithizonates with dilute sulfuric acid solution containing thiosulfate. Thiosulfate and sulfur then have to be destroyed by oxidation before mercury can be re-extracted (*8*).

Mercury can be separated from silver by shaking the solution of their dithizonates with chloride or thiocyanate solution, which transfers the silver to the aqueous phase (*4*).

To determine copper in the presence of silver, silver can be removed by extracting the solution of their dithizonates with iodide (*3*).

GENERAL PRINCIPLES

When the possibility was examined of using ethylenediaminetetraacetic acid as a complexing agent to prevent the extraction of some metals by dithizone, it was found that silver and mercury are extracted in its presence from acid solution but not copper, provided the copper concentration is not too high; otherwise copper is extracted very slowly. If calcium chloride and ammoniacal citrate solution is added to the solution containing copper and ethylenediaminetetraacetic acid, the metal can be extracted by dithizone at pH 9.

Ethylenediaminetetraacetic acid also prevents the extraction by dithizone of lead, zinc, bismuth, cadmium, nickel, cobalt, and thallium at any pH.

The separation of silver from mercury and copper by decomposing its dithizonate by chloride does not allow its subsequent determination by dithizone using existing methods. A way of re-extracting it from chloride solution was therefore needed. It was found that extraction is possible at pH 3 to 3.5. At lower pH extraction is incomplete and at higher pH the color of the silver dithizonate is changed, probably through partial formation of the enol compound. The silver is most easily separated by using a mixture of equal parts of 20% sodium chloride solution and 0.03*N* hydrochloric acid for the decomposition of the dithizonate and re-extracting the silver with dithizone after diluting the aqueous solution to 10 times its volume with water. Alternatively, ammonia can be added to the aqueous silver solution until the pH is 3 to 3.5. The latter method avoids the large volume but is more cumbersome, as the pH limits have to be strictly observed. The danger of incomplete extraction because of too low pH is especially pronounced at a high sodium chloride concentration.

The usual method for decomposing copper dithizonate and transferring the metal to the aqueous phase is rather tedious. It involves evaporation of the organic solvent and destruction of organic matter with sulfuric and perchloric acids (7). In the search for a more rapid way it was found that shaking with 6 N hydrochloric acid brings about complete decomposition of copper as well as mercury dithizonate. After the solution is neutralized with ammonia and acidified to about pH 2, the metals can be re-extracted with dithizone.

REAGENTS

Dithizone solution in carbon tetrachloride, 100 p.p.m. This stock solution is diluted to 13 p.p.m. Concentration is about $0.00005M$, as required.

Sodium chloride solution, 20%, extracted with dithizone.

Hydrochloric acid, $0.03N$ and $6N$.

Ammonia, $6N$.

Hydrochloric acid and ammonia must be freed from copper by distillation or the copper content must be determined and subtracted in each analysis.

Ethylenediaminetetraacetic acid solution, $0.01N$. Dissolve 2 grams of the disodium salt in 1 liter of water. Absence of copper can be checked by adding excess calcium chloride solution (previously extracted with dithizone), followed by ammoniacal citrate solution, and shaking with dithizone solution.

Calcium chloride solution, $0.1N$, extracted with dithizone.

Ammoniacal citrate solution. Dissolve 250 grams of ammonium citrate in water, add ammonia to pH 9, and make up to 1 liter. Extract with dithizone solution and remove excess dithizone by shaking with chloroform.

STANDARD PROCEDURE

The sample solution should have a pH of about 2 if chloride is absent. In the presence of chloride silver cannot be extracted quantitatively at this pH by dithizone. If the chloride content is not over 1%, complete extraction can be achieved at pH 3.5; if it is higher, the pH has to be raised by addition of $0.1N$ ammonia. At pH 5 silver can be extracted quantitatively in the presence of as much as 20% ammonium chloride. It is not advisable to raise the pH far beyond this figure, as an increasing amount of silver enol dithizonate is formed, which is nearly insoluble in carbon tetrachloride.

Extract with successive portions of 13 p.p.m. dithizone solution. If more than 50 γ of metal is expected, especially if the proportion of copper is high, use a stronger solution. When a portion of dithizone solution remains green, discard the aqueous layer. Extract the carbon tetrachloride solution of the dithizonates twice with 3 ml. of a mixture of equal volumes of 20% sodium chloride solution and $0.03N$ hydrochloric acid. The aqueous phase contains the silver.

Determination of Silver. Dilute the aqueous solution containing the silver to 60 ml., extract with 13 p.p.m. dithizone solution, and determine silver by visual mixed-color procedure or photometrically.

Separation of Mercury and Copper. Extract the carbon tetrachloride solution containing the dithizonates of mercury and copper twice with 3 ml. of $6N$ hydrochloric acid. Discard the organic phase. Neutralize the acid with 6 N ammonia and acidify to pH 1.5 to 2. Add 1 ml. of $0.01N$ ethylenediaminetetraacetic acid, more if more than 50 γ of copper is expected.

Determination of Mercury. Extract with 13 p.p.m. dithizone solution, avoiding prolonged shaking, and determine mercury visually or photometrically.

Extraction and Determination of Copper. Add 1 ml. of $0.1N$ calcium chloride solution, 3 ml. of ammoniacal citrate solution, and sufficient ammonia to produce a pH of 9 to the aqueous solution from which the mercury has been extracted, or an aliquot of it containing not more than 20 γ of copper. Extract with successive portions of 13 p.p.m. dithizone solution until a portion no longer shows a purplish color. Wash the combined extracts once with water in order to remove traces of ethylenediaminetetraacetic acid and decompose the copper dithizonate by shaking twice with 3 ml. of $6N$ hydrochloric acid. Neutralize the acid with $6N$ ammonia and acidify to pH 2 to 3. Extract copper with 13 p.p.m. dithizone solution, shaking for 1 minute each time, and determine the metal visually or photometrically.

MODIFIED PROCEDURES

If the amounts of the metals present are vastly different, the procedure has to be modified in most cases. High copper concentrations especially give rise to extraction of some copper in the presence of ethylenediaminetetraacetic acid.

Silver in Presence of Large Amounts of Copper. Add 2 equivalents of ethylenediaminetetraacetic acid, using a strong solution for each equivalent of copper in the sample solution. Extract the silver by shaking with five portions of 13 p.p.m. dithizone solution. Some copper is also extracted. Decompose the silver dithizonate as in the standard procedure and determine silver. The results tend to be low, and only 90% of the silver was found in a mixture of 1 part of silver and 10,000 parts of copper.

Mercury in Presence of Large Amounts of Copper. Extract mercury in the presence of ethylenediaminetetraacetic acid as in the case of silver. Decompose the mercury dithizonate containing some copper dithizonate with $6N$ hydrochloric acid. Neutralize the acid and make pH 1.5 to 2. Add 1 ml. of $0.01N$ ethylenediaminetetraacetic acid and extract the mercury. The method allows the determination of 1 part of mercury in 10,000 parts of copper.

Table I. Recovery of Silver, Mercury, and Copper from Prepared Standard Mixtures

	Silver, γ		Mercury, γ		Copper, γ	
Sample	Taken	Found	Taken	Found	Taken	Found
1	30	28	30	30	20	21.5
2	35	35	25	25	15	14.5
3	15	15.5	10	9.5	50	49
4	20	20	30	33	20	21.5
5	10	10.5	30	29.5	20	20.5
6	30	27	..	..	3,000	Not detd.
7	30	27	30	30	300,000	Not detd.
8	10,000	Not detd.	30	32	10	10.5
9	20	21	1000	Not detd.	10	None

Copper and Mercury in Presence of Large Amounts of Silver. Extract the copper and mercury in the presence of excess sodium chloride at pH 1.5 to 2, disregarding the silver chloride precipitate. Usually the carbon tetrachloride runs off clear, requiring no filtration. Determine copper and mercury by the standard method.

Silver in Presence of Large Amounts of Mercury. The amount of mercury is limited to a few milligrams, because the metal has to be extracted with dithizone. Extract the mercury with strong dithizone solution, 100 p.p.m. or more, in the presence of 10% sodium chloride and $0.02N$ hydrochloric acid. In the aqueous phase determine silver by the standard procedure.

Small amounts of copper in the presence of several milligrams of mercury cannot be determined by this method, as the copper is extracted along with the mercury in spite of the presence of ethylenediaminetetraacetic acid, owing to the high concentration of dithizone required for the extraction of the mercury.

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Received for review September 7, 1954. Accepted November 1, 1954.

Identification of Organic Bases by Means of the Optical Properties of Diliturates (Nitrobarbiturates)

Secondary Aliphatic Amines

ELMER M. PLEIN, *University of Washington, Seattle, Wash.*
BARTLETT T. DEWEY, *Eastern New Mexico University, Portales, N. M.*

The use of the crystallographic and optical properties of the diliturates has been suggested as a means of identification of organic bases by the authors in previous papers (*1, 2*), in which they reported the optical constants for the diliturates of some primary aliphatic amines and primary aromatic amines. The present communication reports similar constants for the diliturates of secondary aliphatic amines.

The methods employed are similar to those used in the earlier works. Equivalent quantities of diliturlc acid (from Eastman Kodak Co.) and the amine were dissolved in a minimum of boiling water and the solution was allowed to cool. Each diliturate was recrystallized one or more times.

The purity of the salts was established by determination of the nitrogen content by the Kjeldahl method modified to include nitro compounds or by the semimicro-Dumas method. All analyses checked with the calculated values within 0.2%, except for the following salts: diallylamine calculated 20.73, found 20.97; diisobutylamine calculated 18.53, found 18.30; diisopropylamine calculated 20.43, found 20.68; α-methylbenzylmonoethanolamine calculated 16.56, found 16.90.

The optical properties for the diliturates of 20 secondary aliphatic amines are given in Table I. The optical properties must be determined on freshly recrystallized samples. Some of the diliturates rapidly lose water of crystallization with accompanying change in their properties.

Diphenylethylenediamine diliturate is the only one of the diliturates which is uniaxial. In the table the values for the indices are indicated as ε and ω.

Diisoamylamine diliturate forms large crystals. When these large crystals are crushed, cleavage occurs parallel to the long axis of the crystal. These lamellar fragments appear like well-formed crystals.

Diethylenediamine shows high dispersion, so that accurate values for the refractive indices are difficult to determine. The value for gamma was determined with the sodium vapor lamp.

Table II records values for optical and crystallographic properties in the most frequently occurring orientation. Most of the diliturates are flattened. Therefore they assume the same or nearly the same orientation when suspended in a refractive index oil. Two of the diliturates present centered figures: Di-*n*-

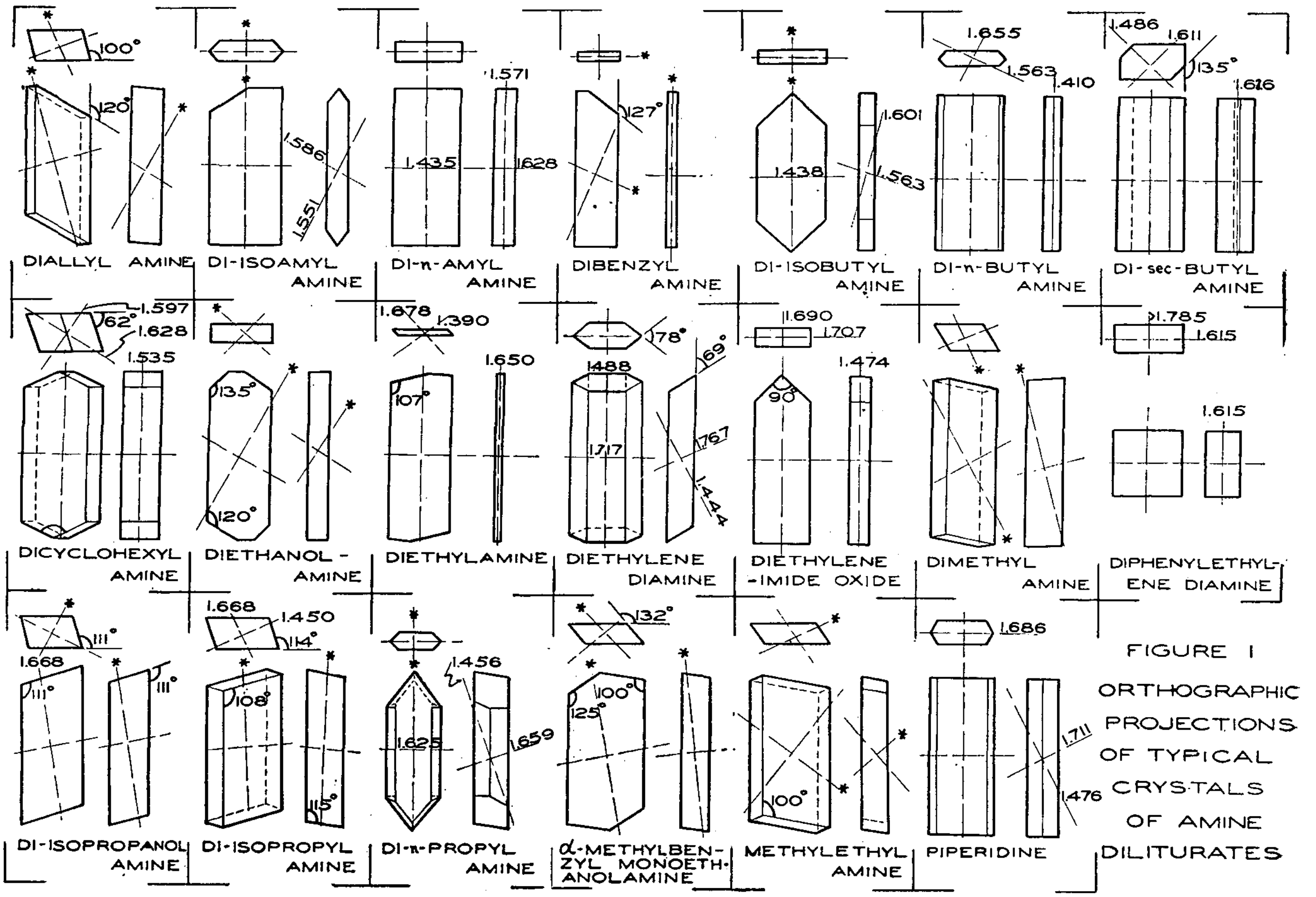

FIGURE I
ORTHOGRAPHIC PROJECTIONS OF TYPICAL CRYSTALS OF AMINE DILITURATES

Table I. Optical Properties of Diliturates of Some Secondary Aliphatic Amines

Diliturate	System	Extinction Angle	Optic Sign	Refractive Indices			Elongation	Dispersion
				Alpha	Beta	Gamma		
Diallylamine	T	27	−	1.461	1.675	1.697	+	$\rho>\nu$
Diisoamylamine	M	25	−	1.452	1.551	1.586	±	$\nu>\rho$
Di-*n*-amylamine	O	0	−	1.435	1.571	1.628	±	$\nu>\rho$
Dibenzylamine	M	26	−	1.548	1.661	1.709	±	$\nu>\rho$
Diisobutylamine	M	21	−	1.438	1.563	1.601	+	$\nu>\rho$
Di-*n*-butylamine	M	28	−	1.410	1.563	1.655	−	$\nu>\rho$
Di-*sec*-butylamine	M	42	−	1.486	1.611	1.626	+	$\rho>\nu$
Dicyclohexylamine	M	28	−	1.535	1.597	1.628	−	$\nu>\rho$
Diethanolamine	T	43	−	1.445	1.624	1.668	±	$\nu>\rho$
Diethylamine	M	35	−	1.390	1.650	1.678	±	$\nu>\rho$
Diethylenediamine	M	28	−	1.444	1.717	1.767	−	$\nu>\rho$
Diethyleneimide oxide	O	0	−	1.474	1.690	1.707	−	$\nu>\rho$
Dimethylamine	T	42	−	1.449	1.722	>1.785	±	$\nu>\rho$
Diphenylethylenediamine	Tet	0	+	1.615(ω)	...	>1.785(ϵ)	−	...
Diisopropanolamine	T	33	+	1.577	1.595	1.673	+	$\nu>\rho$
Diisopropylamine	M	33	−	1.450	1.618	1.668	±	$\nu>\rho$
Di-*n*-propylamine	M	28	−	1.456	1.625	1.659	−	$\nu>\rho$
α-Methylbenzylmonoethanolamine	T	42	−	1.449	1.638	1.705	±	$\nu>\rho$
Methylethamine	T	43	−	1.463	1.700	>1.785	+	$\nu>\rho$
Piperidine	M	27	−	1.476	1.686	1.711	−	$\nu>\rho$

Table II. Apparent Properties of Some Secondary Aliphatic Amine Diliturates from Most Frequently Observed Orientation

Diliturate	Habit	Optical Orientation	Extinction Angle	Refractive Indices	
Diallylamine	Tabular	Inclined optic normal	Variable	Variable	Variable
Diisoamylamine	Tabular	Inclined obtuse	0	1.452	Variable
Di-*n*-amylamine	Lamellar	Obtuse	0	1.435	1.571
Dibenzylamine	Acicular	Inclined acute	Variable	Variable	Variable
Diisobutylamine	Tabular	Inclined optic normal	0	1.438	Variable
Di-*n*-butylamine	Lath	Inclined obtuse	0	1.410	Variable
Di-*sec*-butylamine	Tabular	Inclined obtuse	Variable	Variable	1.626
Dicyclohexylamine	Lath	Inclined optic normal	0	1.535	Variable
Diethanolamine	Tabular	Inclined optic axis	Variable	Variable	Variable
Diethylamine	Lath	Inclined optic axis	Variable	Variable	1.650
Diethylenediamine	Bladed	Inclined obtuse	0	1.488	1.71
Diethyleneimide oxide	Lath	Optic normal	0	1.474	1.707
Dimethylamine	Tabular	Inclined optic axis	Variable	Variable	Variable
Diphenylethylenediamine	Tabular	Inclined optic axis		1.615	1.615
Diisopropanolamine	Tabular	Inclined obtuse	Variable	Variable	1.668
Diisopropylamine	Tabular	Inclined optic axis	0	Variable	Variab
Di-*n*-propylamine	Tabular	Inclined obtuse	0	Variable	1.625
α-Methylbenzylmonoethanolamine	Tabular	Inclined optic axis	Variable	Variable	Variable
Methylethylamine	Tabular	Inclined optic axis	Variable	Variable	Variable
Piperidine	Lamellar	Inclined obtuse	0	Variable	1.686

amylamine diliturate shows an obtuse bisectrix figure and diethyleneimide oxide gives an optic normal figure. Di-*n*-amylamine diliturate shows the true values for alpha and beta, and diethyleneimide oxide the true values for alpha and gamma. The uniaxial crystal, diphenylethylenediamine diliturate, usually exhibits an optic axis figure, so that the index obtained is omega. Diethylenediamine diliturate has an orientation sufficiently constant to show two indices, although one is a value between alpha and beta. In cases where the value for an apparent index varies because of the difference in orientation, the values are indicated as variable.

In Figure 1 are diagrams of the crystals. One view shows the crystal in its most frequently occurring orientation; the others show side and end views. Vibration directions in the crystals are indicated by dotted lines and apparent refractive indices are given. Where no apparent index for a view was obtainable, an asterisk indicates the higher refractive index. The values for prominent angles are given.

The diliturates of diisoamylamine, diisobutylamine, diethanolamine, and diethylenediamine are pleochroic, appearing yellow in the direction of the highest index.

Binary mixtures of some of the amines were used in preparing diliturates. The components of mixtures of diethylenediamine with morpholine (diethyleneimide oxide), dimethylamine with di-*n*-butylamine, diethanolamine with morpholine, and dimethylamine with morpholine could be recognized by the characteristics of the diliturates formed.

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MEETING REPORT

Pittsburgh Conference on Analytical Chemistry

The Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, to be held in Pittsburgh, Pa., February 28 to March 4, is sponsored by the Analytical Chemistry Group, Pittsburgh Section, American Chemical Society, and the Spectroscopy Society of Pittsburgh. It will include an Exposition of Modern Analytical Equipment and visits to several points of interest. Abstracts of the papers to be presented are given here.

Thermogravimetric and Differential Thermal Analyses of Inorganic Perchlorates and Chlorates. Clement Campbell and Saul Gordon, Pyrotechnics Chemical Research Laboratory, Picatinny Arsenal, Dover, N. J.

Thermogravimetric analysis has been greatly advanced in the postwar years with the development of the commercially available Chevenard automatically recording thermobalance. Duval *et al.* have successfully applied this technique to an investigation of the thermal stabilities of insoluble compounds employed in and recommended for gravimetric analysis. However, very little work has been done to utilize this method for a study of the thermolysis of other inorganic compounds, particularly oxidants such as perchlorates, chlorates, and nitrates. The widespread use of these inorganic oxidants in military pyrotechnics, explosives, propellants, smokes, and many other combustible compositions necessitates an investigation of their thermal decomposition, phases of which can be readily accomplished by thermogravimetric analysis.

A complementary technique which has been most widely exploited in ceramic and mineralogical studies is differential thermal analysis. The use of differential thermal analysis in conjunction with thermogravimetric analysis provides a more complete description of the physicochemical reactions accompanying the thermal decomposition of the respective systems under study. In this investigation the thermal decomposition of some perchlorates and chlorates of the Group IA, IB, and IIB elements was studied by means of the combined thermoanalytical techniques. The thermogravimetric analysis and differential thermal analysis curves are presented along with data pertinent to the transition, fusion, and decomposition temperatures, intermediate and final reduction products, and comparisons of the reduction processes. The compounds included are sodium, cesium, copper, zinc, mercury(II), magnesium, calcium, strontium, and ammonium perchlorates and sodium, potassium, and barium chlorates.

Thermogravimetric and Differential Thermal Analyses of Inorganic Nitrates. Saul Gordon and Clement Campbell, Pyrotechnics Chemical Research Laboratory, Picatinny Arsenal, Dover, N. J.

Thermogravimetric analysis and differential thermal analysis are the complementary techniques employed in this investigation of the thermal decomposition of inorganic (heavy metal) nitrates. The thermogravimetric analysis curves obtained with a Chevenard, photographically recording, automatic thermobalance provide gravimetric data for the changes in weight corresponding to the formation of intermediate and final reduction products as a function of time at a linear heating rate of 15° C. per minute. The differential thermal analysis curves, in which differential temperatures (using Al_2O_3 as the reference compound) are recorded as a function of time, with the corresponding sample temperatures indicated at 1-minute intervals are obtained at the same heating rate. These curves consist of inflections (bands, peaks, and pips) corresponding to thermal reactions, both physical—transition, fusion, boiling—and chemical—dehydration, decomposition, oxidation, and reduction. A correlation of thermal phenomena and weight changes obtained on the respective curves permits an evaluation of the sequential decomposition stages and possible intermediate reduction products characteristic of the thermal decomposition of these compounds. The salts included in this investigation are the nitrates of aluminum, cerium(III), chromium(III), cobalt(II), iron(III), lead, nickel, thallium, thorium, and zirconium, and ammonium hexanitratocerate.

Study of the Colorimetric Determination of Platinum with Stannous Chloride. O. I. Milner and G. F. Shipman, Socony-Vacuum Laboratories, Paulsboro, N. J.

A study of the reaction between stannous chloride and chloroplatinic acid indicates that at the low acidity often recommended in the literature, platinum does not form a stannous complex, but rather a "hybrid" complex containing both stannous and stannic tin. This complex is sensitive to slight changes in acidity, time of standing, and concentration of the reacting ions, so that rigorous control of conditions is necessary to ensure reproducible results. At high acidity, a true platinum–stannous chloride complex is formed, which is far more suitable for quantitative photometry.

Spectrophotometric Determination of Cerium. A. M. Feibush and Louis Gordon, Department of Chemistry, Syracuse University, Syracuse 10, N. Y.

Existent methods for trace cerium depend on oxidation of cerium(III) to cerium(IV). Dilute solutions of the latter are unstable. Further, the necessity of measurement in the ultraviolet region precludes the presence of many common ions, thorium, and the rare earths. In addition, the removal of excess oxidant such as persulfate or peroxide is difficult.

In the present method, trivalent cerium is oxidized to the quadrivalent state by lead dioxide in sulfuric acid medium. The excess lead dioxide and the lead sulfate formed are easily filtered off, while the unstable cerium solution is immediately run into excess ferrous ammonium sulfate; the latter is subsequently treated with an excess of 1,10-*o*-phenanthroline and compared to a cerium-free blank prepared in the same manner. The difference in absorbancies measured at 505 mμ is directly proportional to the cerium content.

From 20 to 200 γ of cerium can be determined in the presence of thorium and the rare earths. The method is subject to but few interferences and is rapid, accurate, and sensitive.

***N,N'*-Diphenyl-*p*-phenylenediamine as a Quantitative Photometric Reagent for Copper(II).** R. W. Wise and J. N. Roark, Monsanto Chemical Co., Nitro, W. Va.

An intense blue color was noted when trace quantities of copper(II) were added to acidic solutions of *N,N'*-diphenyl-*p*-phenylenediamine. The feasibility of utilizing this blue copper(II) *N,N'*-diphenyl-*p*-phenylenediamine solution for the quantitative determination of trace quantities of copper was investigated.

The investigation indicated that the blue color resulted from formation of a complex of copper(II) and *N,N'*-diphenyl-*p*-phenylenediamine. The complex is suitable for the quantitative photometric determination of copper in the range of 0 to 10 p.p.m. and is essentially free from interferences from most metals. The colored complex has an absorbance maximum at 685 mμ and is stable for 60 minutes. The absorbance of the complex conforms to Beer's law from 0 to 10 p.p.m. copper.

Field Determination of Microgram Quantities of Niobium in Rocks. F. N. Ward and A. P. Marranzino, U. S. Geological Survey, Denver Federal Center, Denver, Colo.

This relatively simple, rapid, and moderately accurate method for the determination of traces of niobium in igneous rocks is based on the reaction of niobium(V) with thiocyanate ion in a 4M hydrochloric acid and 0.5M tartaric acid medium, after which the complex is extracted with ethyl ether. The proposed procedure is applicable to rocks containing from 50 to 2000 p.p.m. of niobium, and with modifications it can be used on rocks containing larger amounts. Five determinations on two rocks containing 100 p.p.m. or less of niobium agree within 5 p.p.m. of the mean and the confidence limits at the 95% level are, respectively, ±6 and. ±4 p.p.m. The addition of acetone to the ether extract for the niobium thiocyanate inhibits the polymerization of the thiocyanate ion and stabilizes the solution for at least 20 hours.

The proposed procedure permits the determination of 20 γ of niobium in the presence of 1000 γ of either iron, titanium, or uranium; 500 γ of vanadium; or 100 γ of tungsten or molybdenum or both. Data are given to show the applicability of the method to field and routine laboratory use.

Determination of Free Acid in Solutions of Aluminum, Thorium, and Uranyl Nitrates. M. E. Jones, B. F. Rider, and Helen Carach, Knolls Atomic Power Laboratory, General Electric Co., Schenectady, N. Y.

The pH of a near neutral solution of aluminum, thorium, or uranyl nitrate is a sensitive indication of its free acid content. Hence a measurement of pH can form the basis of a free acid determination if the solution contains only one appreciably hydrolyzed constituent and the concentration of this constituent is known. A family of curves of pH *vs.* molarity of salt is plotted. The free acid molarity of an unknown solution is determined by measuring the pH and referring to one of these curves.

The method is simple and can be applied to very small samples with accuracy as good or better than microtitration methods.

Use of Versene in Extractions with 8-Quinolinol. Robert P. Taylor, General Electric Co., Cleveland, Ohio. and N. H. Furman, Princeton University, Princeton, N. J.

Complexing with Versene was studied as a method for improving the selectivity of metal oxinate extraction into chloroform. The usual per cent extraction *vs.* pH curves were determined for a number of metals.

The formation of aluminum, cobalt, iron(III), manganese(II), and nickel Versenates prevents the extraction of these metals below pH 8. However, Versene does not seriously interfere with the extraction into chloroform of molybdenum, titanium, tungsten, and uranium oxinates. A single-stage extraction will remove at least 95% of these metals over some pH range below pH 8 and a quantitative removal is attained by a triple extraction.

Vanadium and copper extract less than 95% below pH 8.0. The extraction of these two metals can be increased to 95% by adding calcium to complex with the excess Versene. Calcium tends to displace aluminum, cobalt, iron, manganese, and nickel from their Versenate complexes, but the resulting drop in pH of incipient oxinate extraction is not sufficient to cause these metals to interfere with the extraction of copper and vanadium.

The absorbancies of chloroform solutions of copper, molybdenum, titanium, uranium, and vanadium oxinates follow Beer's law at least up to a concentration of 0.25 mg. of metal in 25 ml. of chloroform. These metals were determined in a number of synthetic and NBS samples by extracting at the appropriate pH in the presence of either Versene or calcium Versenate and measuring the extract spectrophotometrically.

Separation of Tantalum from Niobium Using *n*-Propyl Arsonic Acid. William A. Dupraw, Armour Research Foundation, Illinois Institute of Technology, Chicago, Ill.

A pyrosulfate fusion of the mixed oxides is dissolved in 3% ammonium oxalate, volume adjusted to 95 ml. and 5 ml. of sulfuric acid added. A 5% aqueous solution of *n*-propyl arsonic acid is added to the boiling solution. The solution is kept hot for 1 hour. The precipitated tantalum compound is filtered, washed with 1% sulfuric acid, and ignited to Ta_2O_5. When the amount of tantalum oxide is less than 10 mg., tannic acid is useful in promoting complete precipitation. When tantalum and niobium are not associated with other hydrolyzable elements, the niobium may be quantitatively recovered by destruction of $C_2O_4^{--}$ with $KMnO_4$, filtration of the hydrated niobic oxide, and ignition to NbO_5.

Determination of Niobium and Tantalum by Multicomponent Spectrophotometry. Warren W. Brandt and David G. Mehrtens, Purdue University, Lafayette, Ind.

Chromotropic acid gives a sensitive color reaction with both niobium(V) and tantalum(V) in concentrated sulfuric acid solution.

The applicability of these systems to the spectrophotometric determination of each element individually has been demonstrated. The molar absorptivity of the niobium system at 485 mμ is approximately 3500. The value varies with the amount of reagent used, but using a large excess of reagent it is possible to get a suitable calibration curve. The molar absorptivity of the tantalum system at 375 mμ is 6600. The absorbance reaches a constant value at a 40 to 1 excess of reagent. Both methods permit reasonable accuracy at 1 p.p.m. concentrations. The colors form rapidly and are stable for several days.

The additivity of the two colored systems has been proved, and simultaneous spectrophotometric determinations have been carried out by measuring at 375 and 485 mμ. This type of application was extended to the simultaneous determination of niobium, tantalum, and titanium.

The interferences are not numerous. Fluoride ion causes only small error. Oxidizing ions interfere, but reduction can be accomplished prior to color formation. Nitrate and borate interfere.

Colorimetric Determination of Niobium in the Presence of Titanium. R. J. MUNDY, Titanium Division, National Lead Co., Sayreville, N. J.

A critical examination of the acetone-intensified thiocyanate procedure was made with the aim of developing a colorimetric procedure for the determination of niobium without the prior time-consuming, tedious, and uncertain separation of titanium. Absorption studies made between wave lengths of 320 and 800 mμ showed that a marked shift occurred in the titanium absorption maximum with change in thiocyanate concentration, whereas the niobium maximum remained constant. The shift in maximum brought about a condition in which total absorption of a mixture of titanium and niobium at 400 mμ was due mainly to niobium, while at 360 mμ the absorption was due mainly to titanium. This condition enabled determination of both niobium and titanium from measurements made at two different wave lengths. The niobium color was not proportional to concentration, but a ratio procedure permitted the determination of the fraction of total absorption contributed by either component at a given wave length. The actual amount was then determined by use of a calibration curve prepared from pure solutions. The absorption spectra of impurities and their effect on the niobium results were also investigated.

Use of Anion Exchange Separations in Metallurgical Analysis. JOHN L. HAGUE, E. E. MACZKOWSKI, AND E. D. BROWN, National Bureau of Standards, Washington, D. C.

Many of the new alloys coming into present-day use contain unusual combinations of elements for which satisfactory analytical methods are not available. Many of these difficult separations can be easily accomplished quantitatively on gram samples by the ion exchange technique using commercially available resins.

The separation of combinations of manganese, nickel, cobalt, and iron occurring in high temperature alloys is illustrated by the use of color slides. Fundamental data for the quantitative separation of mixtures of titanium, tungsten, molybdenum, niobium, and tantalum in hydrochloric-hydrofluoric acid media are given.

The practical use of such separations is demonstrated by the determination of these elements in stabilized stainless and high temperature alloys.

Chemical Analysis of High Purity Metals. OWEN R. GATES, Naval Research Laboratory, Washington, D. C.

To extend the range of accurate analysis of high purity metals, a study has been made of ways by which this can be accomplished chemically. Methods for the determination of the more common contaminants in high purity iron have been investigated.

Various approaches to the solution of general problems which arise in this type of analysis are discussed, and a detailed method is given for the determination of micro amounts of silicon. The iron is separated by means of mercury cathode electrolysis in a polyethylene cell, and the silicon content is established colorimetrically, as the silicomolybdenum blue complex.

The amount of silicon contamination from apparatus, reagents, and water is reported, and optimum conditions for the maintenance of minimum blanks are given.

Turbidimetric Method for the Determination of Selenium in Stainless Steel. W. J. BOYER, Baltimore Works, Armco Steel Corp., Baltimore, Md., AND O. H. FRITZSCHE, Research Laboratories, Armco Steel Corp., Middletown, Ohio.

A turbidimetric method for selenium in stainless steel has been developed. The basis for this method lies in the photometric measurement of the turbidity of the solution resulting from a finely divided suspension of reduced elemental selenium.

Briefly, a 0.10 gram sample is dissolved in a nitric-hydrochloric acid mixture, followed by perchloric acid oxidation to eliminate the carbides present. After oxidation, a dilute hydrochloric acid solution is added and the selenium reduced with stannous chloride. A photometric measurement of the turbidity of the solution before and after filtration yields readings proportional to the amount of selenium present in the sample.

Since the working curves obtained on the Kromatrol, the Beckman Model DU spectrophotometer, and the Klett-Summerson photometer are practically straight lines, an empirical factor may be used. The photometric method has definite advantages in speed over the titration method; a group of six samples may be analyzed in 2 hours as compared to a minimum of 11 hours for the titration method. The reproducibility (2 σ) of the method is indicated to be $\pm 0.01\%$ at 0.20 to 0.30% selenium level.

Direct Photometric Determination of Aluminum in Iron Ores. U. T. HILL, Inland Steel Co., Indiana Harbor, Ind.

A rapid photometric method for aluminum in iron ores is described which does not require the separation of iron or other interfering elements usually present in iron ores.

Iron is complexed with mercaptoacetate prior to the addition of chromoxane cyanine RA. The formation of a colored complex of aluminum is almost instant. A titanium complex, although formed, does not interfere when measured in a narrow band instrument.

Use of an Impregnated Graphite Electrode in Polarography. V. FRANCES GAYLOR, ANNE L. CONRAD, AND JEAN H. LANDERL, Chemical and Physical Research Division, Standard Oil Co., Cleveland, Ohio.

Wax impregnation of a graphite electrode has been found to improve polarographic measurements in several respects. Absorption of wax into the electrode pores greatly reduces the high residual currents normally found with the plain graphite rod. Sensitivity to small concentrations of polarographically active species is thus increased by a factor of 10 or more. In addition, the reproducibility of both current and potential measurements is improved.

The wax-impregnated electrode is applicable to both oxidizable and reducible species. Polarographic data have been obtained on oxidation waves of typical organic compounds of the phenolic, amino, and diamine types, and on typical reduction waves such as those of O_2, Pb^{++}, and Ag^+. Half-wave potentials can be measured with a standard error of less than 0.01 volt. Diffusion currents are proportional to concentration and may be used as a basis for quantitative analytical methods. The standard deviation of current measurements is 3%. Concentrations as low as 10^{-5} *M* can be measured in a stirred test solution.

Polarographic Determination of Antioxidants in Gasoline. V. FRANCES GAYLOR, ANNE L. CONRAD, AND JEAN H. LANDERL, Chemical and Physical Research Division, The Standard Oil Co., Cleveland, Ohio.

A polarographic method for the direct determination of antioxidants in gasoline has been developed which is based upon measurement of the polarographic oxidation waves produced at a wax-impregnated graphite electrode. Analysis is conducted directly on an alcoholic solution of the sample, thus eliminating any preliminary separation steps. The method has already been applied to the determination of two commercial antioxidants, *N*-*n*-butyl-*p*-aminophenol and *N,N'*-di-*sec*-butyl-*p*-phenylenediamine. A single sample can be analyzed in approximately 30 to 45 minutes. Standard deviation of the method is 8%. Concentrations as low as 2 p.p.m. in the gasoline can be determined.

An Automatic *iR* Drop Compensator for Polarographic Use. WARREN JACKSON, JR., Chemical and Physical Research Division, The Standard Oil Co., Cleveland, Ohio, AND PHILIP J. ELVING, University of Michigan, Ann Arbor, Mich.

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. Under these conditions, the experimental polarogram must usually be corrected for the potential (*iR* drop) developed by the cell current flowing through the cell resistance in order to obtain a nondistorted current-potential relation. The correction is not constant for the entire current-potential plot but varies with the increasing cell current.

The automatic *iR* compensator completely eliminates time-consuming manual calculations for the *iR* drop by continuously sensing the cell current and producing 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 graphite electrodes. Reproducibility and accuracy were within the limits of normal polarographic analysis. Measured potential values were accurate to 0.01 volt. In some cases, 40% of the total analysis time can be saved by employing the compensator.

Derivative Polarography, the Two-Electrode Method.

J. Daniel Bode (present address, Fisher Scientific Co., Pittsburgh, Pa.), University of Pittsburgh, Pittsburgh, Pa.

A consideration of the basic two-electrode differentiating circuit of Heyrovský, the curves it produces, and their mathematical analysis. Discussion of a single modification of the circuit and its applicability to practical polarographic analysis.

Current-Scanning Polarography of the Phenylenediamines at Platinum Electrodes.

Ronald E. Parker and Ralph N. Adams, Frick Chemical Laboratory, Princeton University, Princeton, N. J.

The anodic oxidation of the isomeric phenylenediamines was investigated at platinum electrodes. Conventional voltage scanning gave nonreproducible results in agreement with data of other workers. In contrast, the current-scanning technique was found well suited to this study. No fundamental differences between the two methods exist. However, it is believed that more rapid electrode equilibria are established in the current-scanning method, allowing more reproducible results to be obtained.

The rotating platinum electrode was used for quantitative studies. A simple, one-tube current-scanning unit is described. All three phenylenediamines were investigated in a variety of acid, alkali and buffer media. The $E_{1/2}$'s of ortho- and para- vary linearly with pH over a rather wide pH range. The $E_{1/2}$ of the meta wave is pH-independent. Single waves were observed for ortho and para in acetate buffers, while two waves are obtained in strong acid solution. The single wave in acetate buffers was found to correspond to a 2-electron oxidation. Interpretation of the split waves in terms of semiquinone behavior is discussed. While the polarograms in acidic or buffer media precisely identify minute concentrations of the individual isomers, analysis of mixtures is complicated.

Some titrations of the para compound with cerium(IV) using a current-input end point are discussed in terms of the polarographic behavior of the system. The application of current-scanning techniques to the measurement of potentials of labile systems is described.

Effect of the Solvent on the Polarographic Reduction of Cations.

Thomas de Vries and Douglas B. Bruss, Department of Chemistry, Purdue University, Lafayette, Ind.

The effect of solvent on the reduction of metal ions at a dropping mercury electrode was investigated using a silver–silver chloride reference electrode. A variety of cations were studied in water, ethylene glycol, formamide, dimethyl formamide, pyridine, the normal alcohols through pentanol, and isopropyl alcohol.

The half-wave potentials were found to vary in a regular manner with dielectric constant of the solvent, usually becoming more positive as the dielectric constant decreased. Cadmium, chromium, copper, and manganese exhibited an almost linear relation between half-wave potential and dielectric constant. Bismuth, cobalt, nickel, and zinc exhibited a linear behavior in regions of low dielectric constant, but showed curvature or breaks in the regions of higher dielectric constant. Copper exhibited the reverse behavior of the other ions. A correlation was found between the shift in half-wave potential per dielectric unit and the product of the magnetic susceptibility and the reciprocal of the ionic radius.

The variation of the diffusion current with the square root of the fluidity of the solvent clearly demonstrated that, for the majority of the ions, viscosity was not the sole factor affecting the diffusion coefficients. Water, ethylene glycol, and formamide formed one line, which passed through the origin, while the alcohols formed a second line which intercepted the fluidity axis. This second line was parallel to the first for cadmium, chromous, cobaltous, manganese, and nickel ions but divergent for zinc ion and convergent for chromic ion. The experimental data seem to indicate a much larger solvation species in the alcohols for the majority of the ions.

Reductions in pyridine and dimethyl formamide generally gave erratic waves and were not satisfactory. Large irrepressible maxima frequently occurred.

Polarographic Determination of Traces of Molybdenum.

W. Donald Cooke, Baker Laboratory, Cornell University, Ithaca, N. Y.

Molybdenum has been reported to give a catalytic reduction wave in the presence of nitric acid. A modification of this procedure has been studied which has a greater sensitivity and involves fewer interferences. In contrast to most catalytic waves, inert electrolytes are not troublesome and the procedure is highly specific. No direct interferences have been found, although at times separation of the molybdenum is necessary. Ion exchange and extraction methods have been used in the removal of undesirable materials. Typical analysis includes the determination of 0.65 p.p.m. of molybdenum in chromate, 1.0 p.p.m. in permanganate, and 0.5 part per billion in sulfuric acid. Many other types of analyses are included in this study.

Convection Voltammetry. Polarographic Determinations with Platinum and Mercury Convection Electrodes.

I. M. Kolthoff and Joseph Jordan (present address, Pennsylvania State University, State College, Pa.), University of Minnesota, Minneapolis 14, Minn.

Classical polarographic electrodes, such as the dropping mercury electrode and the rotated platinum wire electrode, yield diffusion-controlled limiting currents ("diffusion currents"). Their analytical applicability depends either on calibration with a sample of known concentration of every species which is determined, or on the knowledge of the relevant diffusion coefficients and their quantitative correlation with the corresponding diffusion currents. By working in efficiently stirred systems, conditions were found under which limiting currents are independent of diffusion coefficients and all electroactive species in equinormal solutions yield equal limiting currents when the rate of the electrode process per se is sufficiently large. These currents ("convection currents") are governed by the equation

$$i = nFACT$$

where nF represents the number of coulombs per mole, A the area of the indicator electrode, C the bulk concentration of the electroactive species, and T a "convection coefficient" which depends solely on the hydrodynamic conditions prevailing in the electrolysis cell.

Platinum wire and mercury convection electrodes of simple construction are described and their application to the determination of various electrooxidizable and electroreducible species, in a range of concentrations between 10^{-6} and 10^{-3} M, is discussed. Convection electrodes can be made use of for the determination of trace concentrations of species with small diffusion coefficients, where conventional diffusion voltammetry fails. Convectien voltammetry is also applicable to viscous media, since the viscosity coefficient of convection currents at 25° C. was found to be as small as 0.3% per millipoise in a range of viscosities between 9 and 13 millipoises.

Review of Chemical Analysis Methods for 31 Different Elements in Titanium Metal and Alloys.

Sam Tour and Henry Suss, Sam Tour & Co., Inc., New York 6, N. Y.

The wet chemical and colorimetric methods of analysis for carbon, nitrogen, iron, chromium, manganese, aluminum, molybdenum, vanadium, magnesium, tungsten, chlorine, silicon, nickel, cobalt, copper, zirconium, tin, columbium, tantalum, beryllium, thorium, boron, sodium, phosphorus, sulfur, titanium, calcium, lead, silver, oxygen, and hydrogen, available to date, are briefly outlined.

Gravimetric Determination of Aluminum in Titanium Metal and Alloys.

Sam Tour, Henry Suss, Gregory Pantchenko, and Anna Aronson, Sam Tour & Co., Inc., New York 6, N. Y.

A gravimetric method is available by which aluminum can be determined in titanium metal and alloys in the range of 0.05 to 20%. The procedure is so devised as to eliminate the interference of all possible alloying elements or residuals which could be present in titanium metal or alloys. Titanium, iron, vanadium, tungsten, molybdenum, and zirconium are separated by a cupferron precipitation and a chloroform extraction. Other interfering elements, if present, such as copper, chromium, cobalt, nickel, and the major part of manganese, are separated by the use of a mercury cathode electrolysis. The manganese which remains is precipitated in a sodium hydroxide–peroxide solution. The aluminum is then precipitated with 8-hydroxyquinoline at a pH of 5. The precipitate is filtered, washed, and weighed.

A brief outline is given on results of a cupferron separation of iron and titanium from aluminum. The status of the colorimetric procedures for aluminum in titanium is discussed.

Determination of Gases in Metals by Vacuum Fusion.

Dean I. Walter and Lendell E. Steele, Naval Research Laboratory, Washington, D. C.

Continued emphasis on the importance of very small amounts of gases in metals has prompted the design of a highly versatile vacuum fusion apparatus for the determination of oxygen and hydrogen in such metals as titanium, zirconium, molybdenum, and iron.

Unique features of the apparatus are a simplified catalytic chamber, fractional gas collection and storage facilities, removable furnace, and improved sample loading device.

Data are presented to demonstrate the time-temperature relationship of gas evolution in vacuo from various types of metal samples. The accuracy and reproducibility of vacuum fusion analysis of titanium are demonstrated over the concentration range of 0.005 to 5%; the major limitations of this method are discussed.

Determination of Oxygen in Titanium and Titanium Alloys. Maurice Codell and George Norwitz, Pitman-Dunn Laboratories, Frankford Arsenal, Philadelphia, Pa.

Oxygen in titanium and titanium alloys causes changes in ductility, hardness, and grain refinement; consequently, its determination is of the utmost importance. In this paper a new method is proposed for the determination of oxygen in titanium and titanium alloys. The sample is mixed with carbon and is treated with bromine vapor at 815° C. in a Vycor tube, using helium as a carrier gas. The oxygen in the sample combines with the carbon to form carbon monoxide, which is then oxidized to carbon dioxide with hot copper oxide. The carbon dioxide is absorbed into a weighed bulb containing Ascarite. An investigation was conducted concerning the most practical means for removing the titanium tetrabromide and bromine. The use of an ice bath followed by two Dry Ice–alcohol baths removed most of these substances. To remove the last traces of bromine and bromine compounds, the gases were passed through silver heated to 600° C. A blank of about 0.9 mg. of carbon dioxide per hour (equivalent to about 0.33 mg. of oxygen per hour) was deducted. The average run takes 1.5 to 2 hours. The method differed on the average by 0.007% from the results obtained by the vacuum fusion method, and the standard deviation of the method was 0.009%. The method is applicable to the determination of oxygen in commercial titanium and titanium alloys. This covers the range of about 0.05 to 0.5% oxygen.

Determination of Hydrogen in Titanium and Titanium Alloys. Maurice Codell and George Norwitz, Pitman-Dunn Laboratories, Frankford Arsenal, Philadelphia, Pa.

Hydrogen in titanium and titanium alloys is considered an undesirable impurity; consequently its determination is a matter of importance. The method now being used for the determination of hydrogen in titanium and titanium alloys is the vacuum fusion method which is costly and time-consuming. An investigation was made to develop an inexpensive rapid method for the determination. A thorough survey was made of the methods that have been used for the determination of hydrogen in metals, and the possible application of these methods to titanium is discussed. An ignition method was developed in which the sample is burned in oxygen, the gases are passed through copper oxide to ensure oxidation of the hydrogen to water, and the water collected in a weighted bulb containing anhydrone. A big problem in developing the method was to find means for preventing the large amount of heat generated by the reaction between titanium and oxygen from cracking the tube through which the oxygen passed. The problem was solved by placing the sample in a Vycor tube, 17 mm. in diameter, and supporting this tube in the main reaction tube on clay supports. The method was applied to the determination of hydrogen over the range of 0.005 to 1%. Commercial titanium and titanium alloys contain about 0.005 to 0.02% hydrogen.

Volumetric Determination of Iron in Titanium Metal and Alloys. Sam Tour, Henry Suss, and Gregory Pantchenko, Sam Tour & Co., Inc., New York 6, N. Y.

A rather simple volumetric method is available by which iron can be determined in titanium metal and alloys in concentrations over 1% (possibly as low as 0.25%). The procedure is based on dissolution of the sample in 1 to 1 hydrochloric acid plus some hydrofluoric acid, oxidation of the solution with permanganate, addition of potassium bromide to eliminate the interference of vanadium (if present), and then addition of potassium iodide which reduces the iron from ferric to ferrous with the liberation of iodine. The released iodine is then titrated with standard thiosulfate. The total time for completion of the analysis should not exceed 45 minutes. The elements, except copper, which are normally present in titanium alloys do not interfere. A similar method can be used to analyze for vanadium.

Determination of Micro Amounts of Boron in Uranium Metal and in Uranium Solutions. Katherine Trego and Louis Silverman, North American Aviation, Downey, Calif.

The optimum conditions and amounts of reagents for the colorimetric (curcumin) determination of boron, when present alone, had been established. When uranium is present, the acidity and oxalic acid quantities are disturbed, but related to the amount of uranium present. A new study shows that optimum conditions may be reestablished, and that boron (3 p.p.m. or more) may be determined in the presence of 25 to 100 mg. of uranium in acetone solution.

As a result of this investigation, it is not necessary to make a prior separation of the boron by repeated distillations from uranium solution.

Colorimetric Determination of Higher Concentrations of Uranium in Perchloric Acid Solutions. Lavada Moody and Louis Silverman, North American Aviation, Downey, Calif.

In perchloric acid solutions (2 to 65% by volume), at selected wave lengths, the colorimetric absorbance of uranium solutions is proportional to the concentration. Uranium may therefore be determined colorimetrically as uranium perchlorate, under these conditions. The working range is 10 to 70 mg. of uranium per ml.

Above 80% perchloric acid by volume, the absorbance values deviate from Beer's law, indicating that a new species of uranium is present. The ions are postulated.

Analyses have been performed in solutions containing aluminum, cadmium, iron, thorium, zinc, or zirconium, without interferences.

Nucleonics in Analytical Chemistry. G. W. Leddicotte and S. A. Reynolds, Oak Ridge National Laboratory, Carbide and Carbon Chemicals Co., Oak Ridge, Tenn.

During recent years, certain nuclear phenomena have been used to develop new techniques for chemical analysis. The unique physical properties of nuclear particles, particularly those involving the interactions of neutrons with matter, have become the basis of these methods.

Chief among these new methods is neutron activation analysis. It is now being successfully applied to the determination of microgram and submicrogram concentrations of at least seventy of the elements found in the Periodic Table. The presence of any of these elements in a sample can be determined by measuring the radioactivity induced into a stable isotope, or isotopes, of the element by irradiating the sample with a source of neutrons. The induced radioactivity, as an artificially produced isotope of the element, has its own particular physical characteristics which favor a satisfactory analysis. These characteristics are its rate of radioactive decay and the type of radiations emitted during decay. Neither of these characteristics is ever exactly duplicated in the radioisotopes of another element. Besides this specificity, activation analysis has a great sensitivity of detection for most elements. In some instances, it has been possible to determine certain elements in as small a concentration as 10^{-11} gram. Also, activation analysis is extremely free from the contamination difficulties experienced in most conventional methods of analysis, unless the contaminant has been added to the material before the neutron irradiation.

At the present time, the neutron reactions of the Oak Ridge National Laboratory graphite reactor form the core of an analytical service for the determination of trace elements in many different materials. The fundamentals of the method, its sensitivities, and its applications in this service program are discussed.

In addition to activation analysis, other neutron interactions, involving neutron attenuation measurements, have become the basis for other methods of chemical analysis. Other methods of analysis have become possible through the use of radioactive tracers and such special techniques as autoradiography. The fundamentals of each method, the apparatus used, sensitivites, and present applications are briefly discussed.

Sources of Error in Neutron Activation Analysis. Robert C. Plumb and John E. Lewis, Aluminum Research Laboratories, Aluminum Co. of America, New Kensington, Pa.

The process of neutron activation analysis is considered in some detail, and the general requirements of uniformity of neutron flux, absence of transmutation reactions, and radiochemical purity are pointed out. Experimental results illustrating 16% errors from sample packaging effects, 5% errors from inhomogeneities in the neutron pile, and relatively smaller errors from attenuation of the neutron flux by the samples and neutron shadow effects from neighboring samples are given. Steps are suggested to minimize errors from these sources. The effects of transmutation reactions are illustrated by a specific example, and a method for eliminating them is given. The importance of radiochemical purity and the technique used to determine the purity is illustrated by a case where the insufficient purity caused an error of about 50%.

Application of the Gamma Scintillation Spectrometer to Radiochemical Analysis. Benjamin F. Rider, Knolls Atomic Power Laboratory, General Electric Co., Schenectady, N. Y.

The sodium iodide scintillation counter has made the counting of gamma rays as simple and efficient as the counting of beta rays with

Geiger-Müller tubes. Furthermore, since the complete absorption of a gamma ray within the sodium iodide crystal gives rise to a light flash or scintillation whose intensity is proportional to the gamma ray energy, an analysis of the pulse heights from a phototube observing these scintillations offers a qualitative as well as quantitative analysis of a gamma emitter.

As a result, many gamma emitters can be determined without any chemical separation from a gamma scintillation spectrogram of the liquid or solid sample directly.

The analysis is simplest when gamma absorption is complete and all the energy falls under its photopeak, in which case the area under the peak can be compared directly with the area under the photopeak of a known standard.

However, partial absorption within the crystal, with the escape of a portion of the energy as by Compton scattering, complicates the picture, since the resultant scintillations appear at random lower energies and contribute background under other photopeaks. This Compton scattering can be reduced by use of a large primary crystal from which very few gammas escape, and by surrounding the primary crystal by a secondary crystal which will absorb the scattered radiation. The scintillation in secondary crystals can be used to cancel, by means of an anticoincidence circuit, the scintillation which does not fall under the photopeak.

The spectrograms of a number of individual isotopes and mixtures of isotopes are presented to illustrate the application of this tool to radiochemical analysis, along with a few sketches and drawings of the design of the instrument used by the author.

Activation Analysis of Trace Impurities in Silicon Using Gamma Scintillation Spectrometry. GEORGE H. MORRISON AND JAMES F. COSGROVE, Physics Laboratories, Sylvania Electric Products, Inc., Bayside, N. Y.

The electrical behavior of semiconductor devices, such as transistors, is greatly dependent upon the presence or absence of extremely small amounts of certain elements. In attempting to find an analytical method applicable to the submicrogram range, one is confronted by the limit of detection of the more usual methods, such as absorption and emission spectroscopy. Neutron activation analysis provides the necessary sensitivity for many of the problems involving high purity silicon, and recent developments in the field of gamma scintillation spectrometry have permitted the development of a method for the quantitative determination of most trace impurities in silicon after activation without resorting to chemical separations. The method is described and illustrated with analyses of a number of high purity polycrystalline and single-crystal silicon samples. The accuracy and precision of the method based on analysis of NBS standard samples are presented.

Rapid Radiochemical Determination of Niobium. BENJAMIN F. RIDER, Knolls Atomic Power Laboratory, General Electric Co., Schenectady, N. Y.

The gamma scintillation spectrometer makes possible the determination of fission products by counting the gamma events at their respective photopeaks. In the case of niobium-95, this results in a good separation of niobium-95 activity from all other long-lived fission products except zirconium-95.

A rapid method for the determination of niobium-95 was developed based on gamma spectrometry of a niobic acid precipitate. The solubility of niobic acid in nitric acid was determined as a function of nitric acid molarity in order to select precipitation conditions which give maximum chemical yield for niobium consistent with zirconium-95 decontamination.

It was concluded that high yield and satisfactory separation factors can be obtained by two precipitations of niobic acid with 2 to 3 mg. per ml. of niobium carrier from the oxalate complex in 1*M* nitric acid containing 1 mg. per ml. of zirconium hold back carrier.

An Isotope Dilution–Static Combustion Method for Organic Nitrogen in Milligram Specimens. S. L. JONES AND N. R. TRENNER, Research Laboratories, Merck & Co., Inc., Rahway, N. J.

The progress made during the past decade in the techniques for fractionating and purifying organic compounds on a micro and even submicro scale has not been adequately paralleled by similar advances in the quantitative determination of their elemental composition. In an attempt to close this gap, a technique of analysis based upon the isotope dilution principle and mass spectroscopy and which appears, in theory, to be applicable to submilligram sample sizes is discussed. In particular, its application to the determination of elemental nitrogen is described. No specially constructed instrumentation nor complex gas-handling trains are required. The attainable precision with a 1-mg. sample is about as good as that currently obtainable with the conventional micro-Dumas method when carried out using about 5-mg. samples.

An Isotope Dilution–Static Combustion Method for Organic Carbon in Submilligram Specimens. R. N. BOOS AND N. R. TRENNER, Research Laboratories, Merck & Co., Inc., Rahway, N. J.

Following the analytical principles and techniques enunciated in a previous paper, for the ultramicrodetermination of nitrogen, a method has been developed for the ultramicrodetermination of carbon. In this method C^{13}-labeled succinic acid is used as the source of carbon isotope. Analyses with known compounds are presented demonstrating that the precision of this method, when samples less than 1 mg. are used, is about the same as those obtained with conventional microanalytical procedures using 3- to 5-mg. samples.

X-Ray Methods in Chemical Analysis. H. A. LIEBHAFSKY AND P. D. ZEMANY, Research Laboratory, General Electric Co., Schenectady, N. Y.

Because x-ray methods are still making new friends and being applied to new problems in chemical analysis, it is desirable to survey them briefly indicating their origins, their characteristics, their interrelations, and their present status.

Experimental results bearing on the following topics in x-ray emission spectrography are presented and discussed: (1) precision, (2) limit of detection, (3) correction for background, (4) determination of traces, and (5) new applications.

Recent Advances in Instrumentation for X-Ray Analysis. H FRIEDMAN, L. S. BIRKS, AND E. J. BROOKS, Naval Research Laboratory, Washington, D. C.

The range of x-ray fluorescence analysis has been extended to parts per 10,000,000 by carrying out partial chemical separations and utilizing a focusing spectrograph designed for milligram amounts of sample. Extension of the wave-length range toward the K series of heavier elements has been facilitated by the development of scintillation detectors and a higher voltage (100 kv.) primary x-ray source. In the soft x-ray region, where vacuum or helium path is required, the intensities which are obtainable fall from about 2000 c.p.s. at 5 A. to 200 c.p.s. at 8 A., using currently available x-ray sources and crystals. Greatest room for improvement lies in the x-ray source.

Curved Crystal X-Ray Spectrometers. J. W. KEMP, J. JONES, AND L. ZEITZ, Applied Research Laboratories, Glendale, Calif.

A number of reflecting curved crystal spectrometers have been developed to provide the combination of high speed and high resolution required by modern x-ray fluorescence analysis.

One based upon the (200) atom planes of a lithium fluoride crystal bent to a radius of curvature of 22 inches and crystal surface ground to a radius of curvature of 11 inches covers the range 0.35 to 1 A. in the first order. Another with radii of curvature of 8 and 4 inches, respectively, covers the range 1 to 4 A.

Different types of spectrometers have been designed for different needs. A semifixed-focus type has been developed to allow nesting and the mounting of a group of units in the spectrometer head of the x-ray industrial Quantometer.

Scanning types have likewise been developed which are particularly suited for analytical research work and the solution of a wide range of x-ray fluorescence problems.

The performance of such spectrometers has been studied, particularly from two standpoints. The first has to do with the practical limit of detectability of various elements in various matrices which can be achieved for these curved crystal spectrometers as compared to flat crystal units. The second has to do with the ultimate practical precision which can be obtained by x-ray fluorescence analysis in view of the high intensities made available by curved crystal spectrometers

The New Norelco Autrometer. A. A. STERK, North American Philips Co., Inc., Mount Vernon, N. Y.

The Autrometer, an automatic x-ray spectrometer, is described and performance data are presented. The instrument is built around a new 100-kv. x-ray tube, developed especially for x-ray spectrographic work. A printed record is provided which indicates the ratio of the intensity of the x-ray spectral line from an unknown specimen as compared to the intensity of the same x-ray spectral line from a known external standard. This is made possible by the use of twin beam optics and a beam shutter, the latter being used to permit the use of a single channel detection and amplification—thus only one detector, either a crystal scintillation counter or a proportional counter—is necessary. High-speed counting circuits using decade counting tubes provide a digital output for the printer, and in many cases permit direct readout of percentages of the elements in the unknown sample.

A specially designed goniometer permits the selection of from 1 to 12 different elements in each sample analyzed and indexed through the positions sequentially during each operating cycle.

The instrument uses flat crystal reflection x-ray optics which have been designed for maximum sensitivity. Provision is made for operation either with helium or vacuum in the x-ray optical path to give maximum sensitivity for the longer wave-length x-rays. The instrument is rayproof and has been designed for maximum operator comfort, and is completely automatic except for specimen loading.

Some preliminary results obtained with the instrument are discussed.

Apparatus for Automatic Measurement in Multichannel X-Ray Spectroscopy. I. Adler, U. S. Geological Survey, Washington 25, D. C.

An electronic system consisting of a common high-voltage supply for several Geiger tubes and E1T scalers has been developed. It permits the automatic determination of intensity ratios when used in conjunction with a multichannel fluorescence x-ray spectrograph. A feature of the system is control of one to three scalers by a preselected master scaler with a preset count range from 100 to 1,000,000 in steps of 10. The outstanding advantage is convenience of operation.

The Theoretical Derivation of Fluorescent X-Ray Radiation Intensities from Mixtures. Jacob Sherman, Philadelphia Naval Shipyard, Philadelphia, Pa.

Mathematical expressions are derived for the ratio of intensities of fluorescent x-ray radiation of elements in mixtures of known composition to that of the pure elements. K emission lines only are considered for simplicity. The effect of line intensities due to mutual excitation is the principal factor. The other factor is the polychromatic nature of the exciting beam.

The mathematical deviations can be used to approximate the concentrations of elements in a mixture from measured or given, x-ray intensity ratios.

Determination of Iron by Ultraviolet Spectrophotometry. R. Bastian, R. Weberling, and F. Palilla, Sylvania Electric Products Co., Inc., Kew Gardens, N. Y.

An ultraviolet spectrophotometric method for the determination of iron, which utilizes the absorption of the ferric ion in perchloric acid solution, is presented. The system shows excellent stability, good sensitivity, and adherence to the usual absorption laws. The method has been successfully applied to the determination of iron in electronic nickel and glass samples.

Because no special reagents are needed, the analysis for iron can often be carried out in conjunction with the determination of other elements, and if desired, the entire sample can be recovered and used for subsequent determinations.

Owing to the simplicity and rapidity of the method, it is well suited for routine analyses. The interference of a variety of elements is considered.

Determination of P.P.M. of Iron in Adipic Acid by Ultraviolet Absorption. Richard E. Seeber, Robert G. White, and Kelvin H. Ferber, National Aniline Division, Allied Chemical & Dye Corp., New York, N. Y.

The near ultraviolet absorption of the $FeCl_3$ complex affords a convenient means of determining iron in adipic acid in the range 0.25 to 15 p.p.m.

The use of a predominantly alcoholic solvent system obviates the necessity of carefully controlling chloride ion concentration.

Oxidation with hydrogen peroxide assures that all the iron is present in the ferric state.

The method may be extended to other alcohol-soluble substances having sufficient transparency in the near ultraviolet.

Ultraviolet Spectrophotometric Determination of Bismuth. N. M. Lisicki and D. F. Boltz, Wayne University, Detroit, Mich.

A spectrophotometric study has been made of certain bismuth complexes using a Warren Spectracord. The iodide and the thiourea complexes possess ultraviolet absorbance maxima which can be used as the basis of more sensitive spectrophotometric methods for determining 0.1 to 6 p.p.m. of bismuth. The iodobismuthous acid complex has an absorbance maximum at 337 $m\mu$ with an absorptivity 3.5 times as large as the absorptivity at 465 $m\mu$. The bismuth thiourea complex has an ultraviolet absorbance maximum at 322 $m\mu$ with molar absorptivity of 3.6×10^5 liters per mole cm. The effect of solution variables is discussed.

Dichroism of Small Crystals. Mary L. Willard, John O'Neill (present address, Norwich University, Norwich, Vt.), and Richard Stevens, The Pennsylvania State University, State College, Pa.

The preferential absorption of certain wave lengths of light, in passing through a crystal, is called dichroism when measured in two directions or pleochroism when measured in three directions. This characteristic of colored minerals is not new to the petrographers but more recently has been applied by chemical microscopists to the examination of chemical compounds.

The possibility of more quantitative aspects in the study of dichroism of chemical compounds has led the chemical microscopist to examine the occurrence and cause of dichroism and its usefulness in chemical crystallography. Dichroism is shown to occur in (1) crystals containing oriented dye inclusions, (2) crystals of certain rare earth salts, (3) colored crystals of many coordination compounds, and (4) crystals of many colored organic compounds.

Four methods of measuring the dichroism of small crystals are described and compared: (1) growing small crystals larger, sectioning, orienting, and using Polaroid and spectrophotometer, (2) photographing with microspectrographic ocular in polarizing microscope and measuring the density of the image on the film, (3) orientation of several crystals in the lattice of a plastic and measuring with the spectrophotometer, and (4) measurements based on the projection of the image of a crystal from a polarizing microscope into the slit of a spectrophotometer.

The fourth method of determining the dichroism of a single small crystal presents a new technique. It also furnishes a quantitative method of measuring the dichroism of a single small crystal, a dyed textile fiber, or a dichroic botanical or zoological specimen. This technique for the determination of dichroism is simple, quick, and makes use of standard equipment.

Filter Paper with High Acid and Alkali Resistance. Hanns F. Arledter, Hurlbut Paper Co., South Lee, Mass.

Filter papers of cellulosic or glass nature are not suitable filter media for strong acids and alkalies. Filters made of woven cloth show unsatisfactory, uneven pore sizes.

The investigation deals with new filter paper developments which were manufactured with the help of organic synthetic fibers in random distribution of the nature of tetrafluoroethylene, polyvinyl chloride, and copolymers of vinyl chloride and acrylonitrile or vinyl acetate with high chemical resistance. Such filters were made of 100% organic synthetic fibers alone or in combination with ceramic, quartz, or specific glass fibers.

The filter performance in regard to filter speed, retentivity, pore sizes, temperature limitations, dry and wet strength, and over-all chemical resistance is discussed.

100% Teflon fiber paper resists action by such chemicals or concentrated sulfuric, nitric and hydrochloric acids and aqua regia, as well as concentrated alkalies. It is affected only by fluorine gas and chlorine trifluoride at high temperature and pressure and by molten alkali metals.

Color Comparator for the Determination of Water in Cellophane. Howard A. Scopp and Charles P. Evans, Film Research and Development Department, Olin Mathieson Chemical Corp., New Haven, Conn.

A rapid and simple means is presented for utilizing the classical Karl Fischer method in the determination of the water content of cellophane. The water is extracted from the cellophane by agitation in a suitable solvent. The end point of the titration is determined by viewing a transmitted light beam which passes through the solvent containing methylene blue as an indicator. A visual colorimeter has been developed to facilitate this determination.

The colorimeter consists of an amber glass filter mounted next to a square bottle which is resting on a magnetic stirrer. The amber glass filter serves as a color standard. The square bottle is fitted with a rubber stopper containing holes for a buret, an air inlet tube, and an entrance for samples. A lamp is mounted in the rear of the apparatus, so that the light beam which passes through both the filter and the bottle can be viewed on a ground-glass screen in the front of the apparatus. The solvent, usually methyl alcohol with 2 or 3 drops of methylene blue, is placed in the sample bottle and Karl Fischer reagent is added from the buret until the colors on the viewing screen are matched. The method is estimated to be accurate to within ±4% and reproducible to ±2%. These results are possible through the use of a large sample weight. The method has been used successfully on other materials, such as benzene, polyethylene, and polycaprolactam. However, if the sample to be tested clouds the solvent, the end point cannot be estimated accurately. This has been found to be the case with salts such as barium chloride and sodium tartrate.

Relation between Color and Colorant Composition. Henry Hemmendinger and Hugh R. Davidson, Davidson & Hemmendinger, 76 North Fourth St., Easton, Pa.

The visual observation of color and color differences involves the evaluation of three characteristics of color, such as hue, value, and chroma. The colorist, attempting to control the color of any material has at his disposal three or more colorant composition variables.

If the matching problem is concerned solely with the color under a stated condition of observation, it can be limited to three variables of colorant composition. These might be defined, for instance, in the mixing of pigments, as the ratio of white to total pigment content, the ratio of black to chromatic pigment content, and the ratio of one chromatic pigment to a second. The art of the colorist consists, in part, of his ability to comprehend the relation between the three visually perceived color variables and the analytically controlled colorant composition variables.

Both the description of color and the composition of colorants are amenable to rigorous numerical description, by spectrophotometric and analytical techniques. It is therefore possible to establish graphical systems of analysis which relate color and color difference to composition. This has been done for a particular set of pigments, with the result that the composition corresponding to any color can be rapidly deduced from calibration curves with high accuracy. By making color measurements of a sample to be matched, one can derive the necessary colorant composition from these graphs, and can obtain in a single painting a match within normal commercial color-matching tolerances.

Use of Internal Electrodes with High-Frequency Oscillators. J. C. CLAYTON, J. F. HAZEL, W. M. MCNABB, AND G. L. SCHNABLE, Department of Chemistry, University of Pennsylvania, Philadelphia, Pa.

The present work was undertaken to investigate the feasibility of using internal electrodes in chemical oscillometry. Immersing a set of insulated electrodes in the sample solution is electrically similar to placing the vessel containing the solution between the places of a capacitor. Coated metal and even glass-covered mercury electrodes were found to have sensitive response curves. Some measurements were made with systems in which the sensitive electrode was a test tube containing various amounts of mercury while the grounded electrode was a metal beaker, the inside of which was insulated. The response was for the dilute concentration ranges and the sensitivity varied with the amount of mercury in the electrodes and the thickness of the glass. A submerged capacitor type cell permitted studies on the significance of the physical dimensions of high-frequency cells. Progressively varying the electrode separation distance or the diameter of the glass tube shifted the useful regions of the response curves. Increasing the length of the metal electrodes or decreasing the cell wall thickness magnified the response of the effective concentration regions. Methods of temperature regulation are suggested.

Differential Conductometric Titrations with a Resistance-Capacitance Oscillator Circuit. H. V. MALMSTADT, L. A. KNECHT, AND J. D. WINEFORDNER, Noyes Chemistry Laboratories, University of Illinois, Urbana, Ill.

A conductance titration vessel containing two pairs of platinum wire electrodes was inserted into a resistance-capacitance (Wein bridge) oscillator circuit so that the output frequency was directly proportional to the conductance of the solution.

Various differential conductometric titrations were performed using a constant flow buret and a differential frequency meter. The precision of the differential titration technique was investigated.

Sensitivity of the Bromine-Bromide Potentiometric End Point. WILLIAM C. PURDY AND L. B. ROGERS, Department of Chemistry and Laboratory for Nuclear Science, Massachusetts Institute of Technology, Cambridge, Mass.

The sensitivity of the bromine-bromide potentiometric end point has been investigated with respect to the factors contributing to the reagent blank in a coulometric titration. It has been found that the size of the blank varies directly with the surface area of the indicator electrode and the type of potentiometer used. It also depends upon other experimental factors—i.e., stirring, concentration of reagents, and electrode material and pretreatment. The size of the blank can therefore be diminished by proper choice of experimental measuring conditions. The report that the amperometric detection of coulometric titration end points is more sensitive and more precise than the potentiometric has been confirmed for the system under study.

Cobaltic Oxidimetry. CLARK E. BRICKER AND LARRY J. LOEFFLER, Princeton University, Princeton, N. J.

The extremely high reduction potential of the cobalt(III)-cobalt(II) system (1.8 volts *vs.* the standard hydrogen electrode) is well known but because of the rapid oxidation of water by cobaltic ions, the use of this oxidant as a titrimetric reagent has not been proposed.

Efforts to find a stable cobaltic complex ion which would decompose in acid solution or to prepare a reproducible suspension of cobaltic oxide were unsuccessful. However, it was found that a cobaltic sulfate solution which was prepared by electrolytic oxidation from cobaltous sulfate could be stabilized if kept cold and prepared in the proper acid concentration. Cobaltic sulfate solutions have been kept for more than 3 months without any detectable change in titer. Variations in stability of these solutions with changes in acid concentration cobaltic concentration, and temperature were investigated.

A study was made of the use of cobaltic sulfate solutions for the titrimetric determination of such substances as iron(II), ferrocyanide cerium(III), along with preliminary studies on the titration of arsenite, oxalate, and peroxide. An accuracy of about 1% was achieved in all quantitative determinations. End points of the titrations were detected by spectrophotometric methods.

Standardization of Solutions by Coulometry. EDGAR L. ECKFELDT AND EUGENE R. KUCZYNSKI, Leeds & Northrup Co., Philadelphia, Pa.

Present standardizing techniques have shortcomings which largely result from their inescapable dependency on primary standard substances. The stringent requirements imposed on a substance used as a primary standard and its preparation are well known. In contrast, the coulometric method eliminates the need for primary standard substances and thus offers a promising new approach to the problem of standardizing solutions.

Modern instrumentation makes the electrical measurement of coulombs an accurate and simple operation. The measurement can be referred to standards of high reliability. In the present work the results are based on the volt, ohm, and second, standards which are easily maintained within the limit of error required in quantitative analysis. Conversion from electrical to chemical quantities is given by the Faraday constant.

In the present work the electrical measurements were made with an instrument which was especially designed for coulometric titrations. This instrument supplies a constant and predetermined current to a coulometric titration cell. The current-controlling circuit compares the *RI* drop of a calibrated resistor in the current circuit with the voltage of a standard cell. A timer on the instrument automatically integrates the current and gives a reading directly in microequivalents. The coulometric error in this work did not exceed 0.04%.

Coulometric titrations were carried out on a number of commonly used standard solutions.

The results establish the coulometric method as being well suited for standardizing purposes. In addition to affording high accuracy, this method eliminates the preparation, weighing, and other operations associated with a primary chemical standard. Also, other operations are simplified, as an electrical instrument can be more easily controlled and read than a liquid measuring device. The inherent simplicity and reliability of the instrument greatly facilitated making the electrical measurements.

Improved Conductometric Titration of Weak Bases in Nonaqueous Solvents. JOHN GALT AND W. H. MCCURDY, JR., Princeton University, Princeton, N. J.

Conductometric titrations of weak bases with perchloric acid in nonaqueous solvents generally do not yield sharp end points except for the case where a perchlorate precipitate is formed. The reason for this lies in the fact that the slopes of the titration graph before and after the end point are both positive and nearly the same in magnitude. Investigation of this situation by high-frequency and low-frequency techniques revealed that the slope of the titration graph before the end point can be sufficiently lowered to give a well defined end point.

A study of several solvent mixtures employed to improve potentiometric end-point breaks showed very poor results by the two conductometric methods. The most satisfactory conductometric results were obtained with *p*-dioxane or methyl Cellosolve containing 10% formic acid. This solvent mixture, on the other hand, is not well suited for potentiometric end points. The reaction mechanism of formic acid in this titration procedure is discussed. Results are presented for a number of different types of weak bases incorporating formic acid as a differentiating agent.

The Use of an Anion Exchange Resin for the Separation of Alkali Earths from Alkali Metals. REINOSUKE HARA AND BERT L. VALLEE, Biophysics Research Laboratory, Department of Medicine, Harvard Medical School and Peter Bent Brigham Hospital, Boston, Mass.

Sodium, potassium, calcium, and magnesium play important roles in biological systems. The former two elements generally occur in concentrations far exceeding those of the latter two. This fact complicates their analytical assessment by spectrographic and flame photometric means. These four elements together with phosphorus constitute the bulk of most biological materials, thus also complicating the concentration of other metals present.

A complete separation of sodium and potassium from calcium and magnesium has been achieved by the use of Dowex 1-10 exchange resin in the presence of an ethylene-diaminetetraacetic acid derivative. Furthermore, transition elements have been separated from the above four.

The details of the experimental procedures and their application are discussed.

Chemical Analysis of Thin Films by X-Ray Emission Spectroscopy. Thor N. Rhodin, Jr., Engineering Research Laboratory, Engineering Department, E. I. du Pont de Nemours & Co., Inc., Wilmington, Del.

X-ray emission spectroscopy has been applied to the quantitative analysis of thin (approximately 100 A.) evaporated films of iron, nickel, chromium, and Type 304 stainless steel deposited on Mylar polyester backing. Oxide films associated with passivity of stainless steels isolated from Types 304, 316, and 347 were also studied. The corresponding surface densities of the sample films varied from 1 to 25×10^{-6} gram per sq. cm.

A Norelco basic x-ray generator unit and controls with electronic circuit panel and full voltage stabilization were used. X-rays from a tungsten target were used and the emitted radiation resolved by reflection from a flaw-free lithium fluoride single crystal.

Self-absorption errors for the pure metal films were determined as a function of film thickness and found to be less than 2% for surface film densities the order of 20.0×10^{-6} gram per sq. cm. Absorption errors were also determined as a function of composition for the metals in stainless steel alloys and when combined mechanically by superimposing separate films of the metals in the form of sandwiches. With suitable counting techniques, effective differences in counting rate of 1 count per second were measured with an error of ±5%. Analytical sensitivities for the three metals corresponding to this counting rate are 0.037, 0.061, and 0.175×10^{-6} gram per sq. cm. for nickel, iron, and chromium, respectively.

Comparison of the metallic compositional data obtained by x-ray methods for metal and oxide films 300 A. in thickness or less to those obtained independent by microcolorimetric methods indicate excellent agreement. The conclusion is made that accuracies of 2% or better can be obtained for the chemical analysis of such highly dispersed samples by x-rays without the application of corrections for absorption deviations.

Application of X-Ray Fluorescence to Trace Analysis. Martin B. Cavanagh, Naval Research Laboratory, Washington, D. C.

The elements hafnium, niobium, tantalum, thorium, and uranium, which do not lend themselves readily to spectrochemical or chemical analysis, have been determined in micro quantities in iron by the application of x-ray fluorescence techniques.

The iron is removed by means of a mercury cathode, and the residual elements are concentrated on a Mylar film for x-ray examination.

Five micrograms of these elements can be determined in the presence of each other to an accuracy of 15% of the amount present.

Application of X-Ray Fluorescence Analysis to Ferrous Alloys, Ores, and Slags. J. W. Kemp, J. B. Rittenhouse, and M. F. Hasler, Applied Research Laboratories, Glendale, Calif.

The analysis of a variety of steels and other ferrous alloys has been studied by means of the latest high-precision x-ray fluorescence equipment—the x-ray industrial Quantometer. Quantitative methods have been developed which allow correction for interelement effects, and thus provide results of a high order of accuracy.

The analysis of a variety of ores and slags has likewise been studied. Samples have been analyzed in the "as received" condition, as well as after fluxing and a number of interesting relationships have been found. Attention has been focused on the determination of the ferrous group of elements in these samples.

A Variety of Specific Applications of Fluorescent X-Ray Spectrography. W. J. Campbell and H. F. Carl, U. S. Bureau of Mines, College Park, Md.

A number of papers have been published in recent years on various analyses by means of fluorescent x-ray spectrography. This paper describes several types of applications of this instrument technique in a laboratory handling a wide variety of materials.

The relative merits of wet chemical, optical spectrographic, and x-ray spectrographic analyses are discussed with regard to requirements of sample size and preparation, limits of detection, and accuracy and total time of analyses.

Specific examples of nonroutine applications requiring little or no change in the basic instrumentation are described, as:

The analysis of titanium and titanium alloys for composition, for surface constituents, and for depth of penetration of contaminating elements.

The analysis of major constituents of minerals and ores such as a columbite-tantalite for iron, manganese, niobium, and tantalum without using internal standards, and the rapid analysis of uranium and thorium by the determination of the uranium-thorium ratio correlated with a measure of total radioactivity.

Studies of ferrospinels.

The control of intermediate and final products resulting from pilot plant studies of the recovery of secondary metals.

Rapid nondestructive differentiation between alloys.

Analysis of solutions by characteristic absorption.

X-Ray Fluorescent Analysis of Aluminum Alloys for Copper and Zinc. W. H. Tingle and F. R. Potter, Aluminum Research Laboratories, New Kensington, Pa.

In x-ray fluorescent analysis of multiple component alloys, the influence of extraneous elements is either eliminated by the extensive use of comparison standards or corrected for by calculations involving empirically determined coefficients. In the method presented, only one analytical curve for each element is required, and all absorption coefficients are computed from established principles plus certain appropriate postulates. As the absorption coefficients need not be derived empirically, they can be determined for the minor as well as major components. Absorption of both primary and secondary radiations is considered. The concentration of an element is calculated from the relative intensity of the characteristic radiation and the approximate concentration of extraneous elements. The application and results of this method are presented for the determination of copper and zinc in aluminum alloys.

Precise Determination of the Gasoline Content of Natural Gas. L. V. Guild, Burrell Corp., Pittsburgh, Pa.

A method is described wherein the heavy components in natural gas are determined with a complete breakdown, including isomers through C_5 and the C_6 and heavier components obtained according to carbon atoms. The method is based on the preferential displacement of heavy components from a highly active adsorbent by means of a gas possessing a high adsorption affinity.

A simplified, rapid sampling device is described to aid in the analysis. Accuracy is of the order of 0.02% of the true composition. Analysis time requires 2 to 3 hours.

Determination of Acetylenic Hydrogen by Means of Concentrated Silver Solutions. L. Barnes, Jr., and L. J. Molinini, Research Laboratories, Air Reduction Co., Inc., Murray Hill, N. J.

In connection with an extensive program devoted to the production of derivatives of acetylene, it became necessary to make available a rapid and accurate method for the determination of acetylenic hydrogen. The literature reveals that concentrated solutions of aqueous silver nitrate and silver perchlorate form soluble complexes with acetylene, accompanied by a simultaneous quantitative liberation of hydrogen ions. These observations have been made the basis of a rapid and accurate method for the acidimetric determination of acetylenic hydrogen, employing 2.0 to $3.5M$ aqueous silver nitrate or silver perchlorate as reagents. The procedure has been successfully applied to the analysis of various acetylenic alcohols, hydrocarbons, carboxylic acids, amines, and miscellaneous compounds with an accuracy of the order of ±0.5%. The principal advantages claimed for the method are speed of analysis, a minimum of reagents, and the absence of a precipitate of silver acetylide which greatly facilitates detection of the visual end point. No interference due to halogens or aldehydes has been observed.

Portable Electrochemical Oxygen Indicator. M. G. Jacobson and F. J. DeLuca, J. T. Ryan Memorial Laboratory, Mine Safety Appliances Co., Pittsburgh 8, Pa.

From the theoretical considerations developed in a previous paper [Anal. Chem., 25, 586 (1953)], it was concluded that a cell with a square root response would have several advantages. A new carbon electrode was developed to combine the square root response with maximum electric power output. A number of important improvements were made in the electric circuitry. The construction of the electrode and the mode of its assembly into the cell were greatly simplified. A laboratory model of the complete instrument is demonstrated, and the pertinent performance data are given, illustrated by several slides.

Determination of Trace Amounts of a Carbonyl Compound (Butadiene-Furfural Condensation Product) by Extraction of Its 2,4-Dinitrophenylhydrazone. Paul E. Toren and B. J. Heinrich, Research Division, Phillips Petroleum Co., Bartlesville, Okla.

The butadiene-furfural condensation product in iso-octane solution is reacted with 2,4-dinitrophenylhydrazine in a two-phase system composed of the iso-octane, and the 1:1:2 ethanol-phosphoric acid-water solution which serves as the solvent for the 2,-4-dinitrophenylhydrazine solution. As the 2,4-dinitrophenylhydrazone of the carbonyl compound is formed, it is selectively extracted into the iso-octane phase where its concentration is measured spectrophotometrically at 340 mμ. The reaction requires about 30 minutes. Good results were obtained with solutions containing 5×10^{-6} to $10^{-4}M$ butadiene-furfural condensation product. Preliminary work with other carbonyl compounds indicates that this procedure is applicable to the determination of any carbonyl compound which forms a 2,4-dinitrophenylhydrazone soluble in iso-octane.

Polarographic Determination of Phthalic Anhydride in Alkyd Resins. Paul D. Garn and Esther Wennerblad Halline, Bell Telephone Laboratories, Murray Hill, N. J.

This work was carried out in order to find a simple and convenient method—not subject to interference from other dibasic acids or nitrocellulose—for the determination of total phthalate as phthalic anhydride in alkyd resins and resins solutions. The method consists of saponifying the sample, dissolving the precipitated potassium phthalate alcoholate in aqueous sulfuric acid, and measuring the diffusion current at a dropping mercury electrode. Phthalic acid yields a well defined polarographic wave in aqueous sulfuric acid, pH = 1.5–1.6, when tetramethyl ammonium bromide is the inert electrolyte. The wave height is proportional to the concentration. The half-wave potential is −1.194 volt *vs.* saturated calomel electrode. The diffusion for phthalic acid in this solvent is 8.6×10^{-6} sq. cm. sec.^{-1} The method permits determination of phthalate more quickly than by simple precipitation of potassium phthalate alcoholate and without significant interference from other dibasic acids or nitrocellulose.

A Microanalytical Procedure for the Determination of Decaborane. William H. Hill and Marian S. Johnston, Graduate School of Public Health, University of Pittsburgh, Pittsburgh, Pa.

Analytical procedures used in the past for the determination of boranes were based on the hydrolysis or oxidation of these compounds to boric acid. The method described in this paper permits the direct determination of decaborane by measuring its ultraviolet absorption in alkaline media at a wave length of 270 mμ, triethanolamine being the preferred reagent. Absorption follows Beer's law. Accuracies of a few per cent are attained easily. The new method is applicable especially to dynamic air analysis.

Nitro Analysis. A New Method for the Determination of Aliphatic Nitro Compounds. Ronald L. Sweet, Roderick S. Spindt, and Vincent D. Meyer, Gulf Research & Development Company's Multiple Fellowship, Mellon Institute, Pittsburgh, Pa.

Although a number of methods for determining aromatic nitro compounds are available, no method of general applicability has heretofore been described for the quantitative determination of the nitro group in aliphatic compounds. The nitroparaffins and their derivatives have now, however, become commercially available. This paper describes a method for determining primary and secondary nitroparaffins, involving treatment of the compounds with hydrogen peroxide and base. Under specified conditions of concentration and heating, the nitro group is converted quantitatively into nitrite ion, which is then determined colorimetrically after reaction with Griess-Ilosvay reagent. Mononitroaromatics, amides, aromatic amines, most aliphatic amines, aliphatic nitriles, derivatives of hydrazine, and nitrogen-containing heterocycles such as pyridine, do not ordinarily interfere with the test within the limits of accuracy. Polynitroaromatics, nitrite esters, and some derivatives of hydroxylamine may interfere to some extent.

Interlaboratory Evaluation of Analytical Methods. W. J. Youden, National Bureau of Standards, Washington, D. C.

The results of interlaboratory test programs are often disappointing because they reveal only what was known before—namely, that determinations in different laboratories show much greater disagreement than determinations made in the same laboratory. This paper takes up the proper scope and objectives of interlaboratory programs. The statistical methods used in the interpretation of the results are illustrated using data obtained in an interlaboratory investigation.

Industrial Statistics—Something New in the Scientist's Tool Kit. Edwin J. Newchurch, Esso Laboratories, Esso Standard Oil Co., Baton Rouge, La.

Modern technology has matured to the point that new developments need to be evaluated even more precisely than in the past. This means that the scientist's tools (analytical methods, pilot plants,
etc.) which are used to measure differences must be sharper–
more accurate and more precise. This situation has given rise t
development of a relatively new science on the basis of a relativel
concept. This new science, called "industrial statistics," is
around the observation that the variability experienced in tech
work follows a definite pattern—that of the normal or Gaussiar
tribution. If the modern scientist will (1) acknowledge the exis
of random error, (2) understand the characteristics of the norma
tribution, and (3) apply a few simple tools which follow logically
this understanding, he can greatly increase his output for a give
penditure of effort.

Experimental Designs in Investigations of Homogeneity of Al
Bourdon F. Scribner and Robert E. Michaelis, National Bu
of Standards, Washington, D. C.

Attention to the design of experiments is essential in order to o
the maximum of information with the minimum of effort, and to
vide optimum arrangements for statistical treatment. Severa
signs employed in studying the homogeneity of alloys intend
spectrographic standard samples are outlined and the reducti
the experimental data by variance analysis is described. Includ
the problems are steel-, tin-, and zinc-base alloys prepared
different metallurgical procedures. The testing of a statistical t
ment to determine its sensitivity for the desired tests and mod
tions of the treatment to obtain greater sensitivity are importa
the application of statistics. The complete analysis of variance fo
data from sets of alloys for several elements is a laborious task
can be eased by machine methods These as well as short-cut
cedures for rapid inspection of repeatability data are discussed.

Chemical Applications of High Resolution Nuclear Magnetic
nance Spectroscopy. James N. Shoolery, Varian Associates,
Alto, Calif.

The theory of nuclear magnetic resonance is reviewed briefl
the instrumentation problems are discussed. The concept of
tronic magnetic shielding of the nucleus is introduced and its rel
to the molecular structure considered. Examples of the applicati
the technique to the solution of chemical problems, both in the fi
organic and fluorocarbon chemistry, are given.

Quantitative Determination of Elemental Hydrogen by Nu
Magnetic Resonance. R. B. Williams, Humble Oil & Refining
Baytown, Tex.

A method has been developed for determining total elementa
drogen in petroleum hydrocarbon samples. The NMR spectro
is operated in the dispersion mode, and a relatively high *rf* pov
used so that the spectral band width is broadened and made
compared to random fluctuations in the static magnetic field.
static magnetic field of the spectrometer is modulated with a
soidal field of amplitude comparable to the magnitude of the
width of the dispersion band, and the fundamental of the modu
component of signal is amplified, detected, and recorded on a pen
ing strip chart recorder. Scanning is achieved by varying the
magnetic field component from a lower to higher field across the
tion of resonance. The rate of scan is as rapid as the response
of the recording apparatus will allow. The period of the modul
frequency (80 c.p.s.) usually is less than the spin-lattice relax
times of the samples. When these conditions are met, the rel
signal intensities per unit hydrogen concentration vary by no
than about 5% although relaxation times of various samples on
the method has been employed vary by as much as a factor o
Thus far, no correction for this small variation based on relax
time measurements has been made. Analytical precision an
curacy for a wide variety of petroleum fractions are discussed.

A New Method for Measuring Magnetic Susceptibilities.
Reilly, H. M. McConnell, and R. G. Meisenheimer, Shell
velopment Co., Emeryville, Calif.

The method requires the use of a high resolution NMR spect
eter. The sample cell consists of two concentric, cylindrical
silicate glass tubes arranged so as to produce an annulus about 0.2
thick. It is placed in the NMR spectrometer so that the co
axis of the tubes is perpendicular to both the static (H_0) and radi
quency magnetic fields. If a sample of water, for example, is put i
annulus of the cell, the single, sharp resonance normally found w
cylindrical sample is seen to be broadened and to consist of two
separated by a lower plateau. Both experiment and theory ind
that the peak separation ΔH is given by

$$\Delta H = 4\pi H_0\left[(K_1 - K_2)\left(\frac{a}{r}\right)_2 + (K_2 - K_3)\left(\frac{b}{r}\right)^2\right]$$

where a, b are the internal and external radii of the inner borosil
glass tube; r is the mean radius of the annulus; K_1, K_2, and K

olume magnetic susceptibilities of (1) the material contained in ner borosilicate glass tube, (2) borosilicate glass, and (3) the an- liquid.

is is a fast and convenient method for the measurement of mag- susceptibilities, which are needed when corrections to NMR ra for bulk magnetic shielding effects are important.

in-Spin Coupling Studies of the Effect of Substituents in the ene Ring. EDWARD B. BAKER, The Dow Chemical Co., Midland, .

has been observed that the F^{19} line in fluoroethylbenzene and obenzene is split into 5 and 6 components, respectively, and that H^1 resonance is a double line. These data indicate that with the r F^{19} substituent the benzene hydrogens are still all chemically valent and act as spin groups of 2 and 5/2, respectively. Relative sities follow Gutowsky's rule. This is in contradiction to existing ical ideas. In *p*-nitrocumene, however, the benzene hydrogens ot chemically equivalent and instead split each other, giving a acteristic four line pattern. Quantitative values of δ and J are .

e Reaction of Oxygen in a Mass Spectrometer to Form Carbon oxide. G. F. CRABLE AND N. F. KERR, Gulf Research & De- pment Co., Pittsburgh, Pa.

investigation has been made of the formation of carbon mon- e by the reaction of oxygen with carbonaceous materials in the ion ber of a mass spectrometer. A special examination of the oxygen nfrared absorption showed less than 0.1 mole % carbon mon- e, while a mass spectrometric analysis indicated 5 mole % carbon oxide. The carbon monoxide production was found to be directly ortional to the oxygen pressure in pure oxygen samples and ds of oxygen and argon and oxygen and normal butane.

survey of previous oxygen calibration data showed variations in atio of the quantity of carbon monoxide formed to oxygen pres- . These variations depend on the condition of the ion source at time of the measurement. Oxygen runs made with a new fila- t that had never been exposed to hydrocarbons showed a 75% ease in the carbon monoxide to oxygen ratio. Passing 2-butene hours over a filament which had been in continuous use process- ydrocarbon samples for 10 months caused a 20% increase in the on monoxide to oxygen ratio. It is believed that the carbon oxide is formed by the oxygen decarbiding the tungsten fila- .

ass Spectra of Trimethylsilyl Derivatives. A. G. SHARKEY, JR., LEY H. LANGER, AND R. A. FRIEDEL, Synthetic Fuels Research ch, U. S. Bureau of Mines, Bruceton, Pa.

e mass spectra of 8 trimethylsilyl normal aliphatic ethers and ral related silicon compounds have been obtained. These com- ds have higher volatility than hydrocarbon and oxygenated com- ds containing a corresponding number of carbon atoms. As a re- the mass spectrum of a trimethylsilyl ether containing 13 carbon s can be obtained without difficulty using a conventional room erature mass spectrometer. This cannot be done with the cor- onding C_{10} alcohol.

imethylsilyl ethers show several intense rearrangement peaks in tion to the expected fragmentation peaks. Numerous other in- ces of rearrangement peaks occurring in mass spectra have been rted, mainly for oxygen-containing compounds. Earlier work on nilar silicon compound, $(CH_3)_6(Si)_2O$, also indicates many re- ngement peaks. An attempt has been made to identify the ture of these rearrangement ions.

eliminary work on synthetic blends indicates mixtures of tri- ylsilyl ethers can be analyzed in the presence of higher molecular ht hydrocarbons. Major fragmentation peaks from silyl ethers ree of interference from hydrocarbon-type ions.

evelopment and Application of the Pressing Technique to Single Multicomponent Systems. ULRICH SCHIEDT AND HELMUTH WEIN, University of Pennsylvania, Philadelphia, Pa.

e development of two pressing dies for quantitative work is de- ed. The semimicro instrument requires 0.5 to 0.8 mg. of sub- ce, the micro die 0.05 to 0.08 mg. The average particle diameter been found to affect the accuracy of intensity measurements to a iderable extent. The effect of the particle size on frequency, in- ity, and half width of absorption bands is discussed. These ef- become negligible if the particle size is very small as compared the wave length of transradiated light. Particles with 200 A. eter can be obtained by lyophilization of aqueous solutions. The ensions of such particles in potassium bromide behave like true tions. Using these methods a determination of aromatic amino s either in pure state or in mixtures has been possible with an age error of ±1%. The technique can be applied to determine true integrated absorption intensities of solid materials. The combination of paper chromatographic separation and infrared photometry using the potassium bromide technique is illustrated by a recently developed method for determination of chain length of peptides.

Infrared Studies of Salts of Some Saturated and Unsaturated Acids. SISTER MARY LAWRENCE O'DONNELL, Mount Mercy College, AND SISTER MIRIAM MICHAEL STIMSON, O.P., Siena Heights College, Adrian, Mich.

The infrared spectra of a variety of carboxylate salts have been studied in potassium bromide disks.

In the ammonium salts not only do the NH_4^+ and COO^- bands appear, but the C=O band associated with un-ionized COOH continues to occur, although in decreased intensity.

In the monocarboxylic unsaturated acids the C=C stretching band is shown up very sharply for the alkali salts. On the other hand, the C=C band is not exposed in the salts of maleic acid.

In salts where a metallic cation is employed the COO^- vibration is sensitive to cation formation, and to water of hydration.

An Infrared Study of the Metal Chelates of Some Imidazole Derivatives. T. R. HARKINS, J. L. WALTER, O. E. HARRIS, AND H. FREISER, Department of Chemistry, University of Pittsburgh, Pittsburgh, Pa.

Infrared absorption curves have been obtained in the 3–13-micron region for a series of metal chelates of 2-(2-pyridyl)- and 2-(*o*-hydroxyphenyl)-derivatives of benzimidazole, imidazoline, benzoxazole, and benzothiazole. Use was made of the potassium bromide pressed pellet technique for the preparation of samples.

Particular attention has been focused on the 3-micron region in order to observe the effects of coordination on the N—H stretching frequency in the benzimidazole and imidazoline derivatives. 2-(*o*-Hydroxyphenyl) benzoxazole and its chelates have an absorption band at 8.0 microns which undergoes a regular shift paralleling the usual stability order of the metals. The copper(II) chelate is at the highest frequency.

Infrared Spectra of Some Metal-Organic Complexes. GERALD D. GRANT AND A. LEE SMITH, Dow Corning Corp., Midland, Mich.

Many analytically important metal-organic complexes have not been previously studied by infrared spectroscopy. We have examined the spectra of the metal complexes of 8-quinolinol, cupferron, and several other complexing agents using the potassium bromide pellet technique. The spectra of 30 8-quinolinols are all different, suggesting an application to qualitative and possibly quantitative analysis of pure materials and of mixtures.

Infrared Study of a Solid Phase Reaction between Thiourea and Some Alkali Halides. JAMES E. STEWART, National Bureau of Standards, Washington, D. C.

The infrared absorption spectrum of thiourea in pressed disks of sodium bromide, sodium chloride, or potassium chloride is the same as the spectra of suspensions in liquid media—e.g., Nujol. But when thiourea is pressed in pellets of potassium bromide, potassium iodide, or cesium bromide its spectrum changes in a way depending on both the alkali metal and the halogen ions. The spectrum of each of the new products is the same as that of the complex which forms when thiourea is crystallized from a solution containing the corresponding alkali halide. In the reaction product spectra certain of the skeletal vibration bands of thiourea are split into two or three components, although the N—H bond stretching vibrations are not altered. A tentative explanation of the reaction can be given involving coordination of the alkali metal ion and sulfur which disturbs the electron distribution of the C—N and C—S bonds. The mechanism requires rupture of crystal lattices followed by diffusion. The more tightly bound sodium bromide, sodium chloride, and potassium chloride crystals are not ruptured in the low pressure die used to prepare pellets so the reaction does not occur even though complexes with these substances form in solution.

Infrared Spectra and Atomic Arrangement in Soda Borate Glasses. ROBERT L. BOHON, SCOTT ANDERSON, AND DUDLEY KIMPTON, The Anderson Physical Laboratory, Champaign, Ill.

Infrared reflection and transmission spectra using the potassium bromide pellet technique of a series of soda borate glasses are presented. The relative merits of the two methods of securing useful data are discussed briefly. The pellet technique permits the detection of trace impurities, such as water, which may influence the atomic arrangement and yet not be detected by reflection spectra. Because of the vibration damping effected by those ions serving as glass modifiers, neither type of spectra is completely amenable to an unambiguous interpretation. Nevertheless, an attempt is made to correlate available data relative to the atomic arrangement in soda borate glasses and pure B_2O_3 glass. The general conclusion is that there is no single class of coordinated arrangement present in any of the glasses. Even in the case of the pure B_2O_3 the usual assumption of 100% triangular-coordination is shown to require modification.

Quantitative X-Ray Fluorescence Analysis of Glass. J. F. BACON AND V. POPOFF, Research & Development Laboratory, Corning Glass Works, Corning, N. Y.

This paper outlines the methods developed at the Corning laboratory for the quantitative x-ray fluorescence analysis of glass.

The basic theory of fluorescence analysis is reviewed and the advantages and disadvantages of the method are compared to other methods.

The authors have worked with both the curved mica transmission crystal type spectrograph and the flat single-crystal type spectrograph. Results obtained with both types of spectrograph, are included. Detectors used were neon-, argon-, and krypton-filled Geiger counters, as well as argon- and krypton-filled proportional counters and a sodium iodide scintillator. The paper compares the response of these detectors as a function of atomic number.

Data are included to show the applicability of the method for the analysis of different elements in glass including Cd, Zn, Se, Zr, Ag, Fe, Sb, As, Rh, Pt, Ba, K, S, Ca, Ti, and others. The precision of the method which is a function of the total count, is discussed. For large concentrations, when the background can be neglected the relative probable error is ±0.51%, while for smaller concentrations the background serves to limit the precision.

The questions of sample preparation and sample thickness are briefly considered.

X-Ray Fluorescence Determination of Zirconium and Titanium Oxides in Glass. J. W. TIMS, Owens-Corning Fiberglas Corp., Newark, Ohio.

A method is presented for determination of zirconium and titanium in glasses. This method involves the use of the latest x-ray fluorescence techniques.

Zirconium-titanium glasses are used as internal standards. The intensity of the zirconium and titanium spectra is measured and related to standard glasses. These intensity ratios are then converted to concentrations. The concentration limit in this type of glass is from 2.0 to 10% zirconia, and from 3.0 to 10% titania. The standard deviation is approximately ±0.2 with a concentration of 10.0% of either element. Single determinations can be made in 20 minutes.

Analysis for Rubidium and Cesium by X-Ray Spectroscopy. I. ADLER AND J. M. AXELROD, U. S. Geological Survey, Washington 25, D. C.

Analyses for rubidium and cesium by wet chemical methods are lengthy. X-ray spectroscopy can analyze rocks quantitatively and quickly, detecting rubidium down to 0.02% and cesium down to 0.1%. Though the cesium spectra are in unfavorable regions for measurement by most x-ray fluorescence spectrographs, routine and dependable methods for the determination of both cesium and rubidium have been worked out. The apparatus in use is described.

Determination of Germanium by Fluorescent X-Ray Spectroscopy. W. J. CAMPBELL AND H. F. CARL, U. S. Bureau of Mines, AND C. E. WHITE, University of Maryland, College Park, Md.

Starting with the basic relationship between the intensity of a spectral line and the concentration of the analytical element the following expression has been derived:

$$I_{\mathrm{Ge}K\alpha} = \frac{KN_{\mathrm{Ge}}}{\mu_E + \mu_{\mathrm{Ge}K\alpha}} \tag{1}$$

where N_{Ge} = concentration of germanium in sample

μ_E = linear absorption coefficient of matrix for wave lengths that excite germanium. The effective value of λ_E was considered to be 1.0 A.

$_{\mathrm{Ge}K\alpha}$ = linear absorption coefficient of matrix for Geα radiation

Three general methods of analysis are described, using the above expression.

The first involves a comparison of the relative intensities of Ge$K\alpha$ lines from unknown and standard samples. This method is limited by the suitability of available standards.

An additive technique was developed in which the intensity of Ge$K\alpha$ is measured both before and after addition of a known amount of GeO_2. This method is based on the assumption that $\Sigma(\mu_E + \mu_{\mathrm{Ge}K\alpha})$ remains practically constant after small additions of GeO_2. Errors due to variation in absorption coefficients are discussed with respect to practical concentration ranges.

The third method is an internal standard technique using as standards, elements having characteristic wave lengths approximately equal to Ge$K\alpha$. General matrix effects on the line ratios with regard to mutual and preferential absorption and preferential excitation are discussed in detail. Also errors due to a variable voltage and to counter tube nonlinearity and particle size effects are considered.

These three methods were used in the study of germanium in coals and coal ashes. The sensitivity of detection and accuracy of analysis are compared to chemical and optical spectrographic methods. These x-ray techniques were used to investigate the reported loss of germanium in coals upon ashing. The effects of the time and temperature of ashing, of ashing agents, of sample size and area, and of available oxygen are described.

Applications of the General Electric X-Ray Photometer in a Petroleum Laboratory. H. D. TERRELL AND J. C. DAVIDSON, Refinery Technology Laboratory, Gulf Oil Corp., Philadelphia, Pa.

The x-ray photometer has proved to be a valuable tool for the control testing of petroleum products, not only in the time saved in laboratory testing, but also in the increase in plant operating efficiency due to the much shorter time that compounding and storage facilities are tied up while control testing is being performed.

X-ray photometric techniques have been applied to the determination of the tetraethyllead content of gasoline, the additive content of oils and sulfur in various petroleum products. While the method is not specific for any of these determinations, the results are very reliable if the technique is properly applied. Tetraethyllead determinations can be made in 5 minutes and additive determinations in 5 to 10 minutes, depending upon the nature of the material being tested.

X-Ray Spectrometric Determination of Zinc in Aqueous Solutions. G. H. JOHNSON AND R. E. WISCHMEIER, Central Research Division, Ferro Corp., Cleveland 5, Ohio.

A technique for the determination of zinc in aqueous solutions using the fluorescent x-ray spectrometer is described. Emphasis is placed on general techniques rather than on the determination of zinc in a specific type of sample. Although zinc is very well suited for determination in this manner, the method is applicable to many other elements detectable with commercial x-ray spectrometers.

Recent Advances in Mineral Analysis by Fluorescent X-Ray Spectrography. MERLYN L. SALMON AND JAMES P. BLACKLEDGE, Denver Research Institute, University of Denver, Denver 10, Colo.

On the basis of the consolidation of results from the literature, fundamental studies were undertaken to determine the effects of experimental variables such as sample quantity, thickness of the sample, area of the sample, operating power of the x-ray tube, wave length of primary x-radiation (tungsten or molybdenum targets), the use of filters on the x-ray tube, and other miscellaneous factors in the analysis of minerals.

Fundamental design factors were then determined for modification of the standard commercial equipment to improve the analytical techniques and results.

Several mineral systems, including naturally occurring and synthetically compounded specimens, were investigated with respect to x-ray absorption characteristic of the matrices and relative interference conditions in fluorescent x-ray spectrography.

Arithmometry, a New Technique for Particle Counting. FREDERICK BRECH, Jarrell-Ash Co., Newtonville, Mass., AND ALAN RICHARDSON JONES, Blood Grouping Laboratory, Boston, Mass.

A new instrument has been developed to provide an accurate particle counting in given size ranges. The accuracy of 3% is independent of the shape or size of particles within the given limits. The instrument comprises a light source, a scanning stage, a simple fixed focus microscope, an aperture system, photomultiplier, and scaling circuits. The sample carried on a stage illuminated by a dark field system is scanned by the microscope objectives.

A sample maintained in a suitable suspension fills a counting chamber which locates on the stage of the instrument in the path of a built-in microscope provided with dark field illumination. An image of the cells is formed in the plane of a precision aperture in front of a photomultiplier. A motor drive unit causes the area of the counting chamber to be scanned by the light beam. Cells whose image is wholly within the precision aperture and those only partly within it are counted. Compensation for edge cells is given automatically during a second stage of the scanning cycle during which time an aperture of modified dimensions is employed. The photomultiplier detects the image of each cell that passes across its photo cathode and the resulting pulses of electronic energy are fed into a simple computer circuit designed around the use of glow transfer tubes.

Application of the new instrument and the new technique is described.

Cyanogen-Oxygen Flame as a Spectroscopic Source. Bert L. Vallee and Milton R. Baker, Biophysics Research Laboratory, Department of Medicine, Harvard Medical School, and Peter Bent Brigham Hospital, Boston, Mass.

The use of flames in spectrochemical analysis has been applied widely to the analysis of the alkali metals and alkaline earths. For these elements the flame is a particularly suitable source, yielding data of high precision, accuracy, and sensitivity. Thus, it compares favorably with arc or spark sources. However, even hydrogen-oxygen flames fail to excite energy levels of most of the other elements, limiting the range of application of the flame as a spectroscopic source.

The cyanogen-oxygen flame with an approximate temperature of 4700° K. has been studied in this laboratory as a source of spectral excitation. In this flame the spectra of many elements are excited where under similar conditions they cannot be discerned in an oxygen-hydrogen flame. This source shows great promise for analytical spectroscopy. Details of the findings are reported.

A Multichannel Flame Spectrometer Employing Automatic Background Correction. Marvin Margoshes and Bert L. Vallee, Biophysics Research Laboratory, Department of Medicine, Harvard Medical School, and Peter Bent Brigham Hospital, Boston, Mass.

It has been reported that the analysis of solutions containing alkali metals and alkaline earths is complicated by the presence of background or noise. The background increases in direct proportion to the concentration of each radiating species present. This heterochromatic light appears as noise at the analytical wave length of the element to be determined. To obtain accurate analytical data, it is necessary to correct for this background.

A multichannel flame spectrometer, using a grating monochromator and separate exit slits and detectors for sodium, potassium, calcium, and strontium, has been modified to include automatic correction for the background. An auxiliary exit slit is placed $^1/_4$ inch to one side of the main exit slit for each analysis line of an element. Since the photomultipliers cannot be placed sufficiently close to one another, front surface mirrors are used to reflect the light onto a photomultiplier placed slightly to the side and to the rear of the exit slit. The signals from the photomultipliers, placed on the line and background exit slits, respectively, are amplified separately. The amplified signals are subtracted electrically before being presented on a microammeter. The meter thus indicates the difference between line-plus-background and background—i.e., the corrected line intensity.

This system of background correction makes possible the direct determination of one element in the presence of considerably larger concentrations of other elements. The quality of the analytical results is a function of the line to background ratio. Good precision is obtained when background intensity is as much as seven times the value of the line intensity.

Determination of Dissolved Solids in Water Samples by Flame Photometer. Grace E. Marsh, Research Department, Standard Oil Co. (Indiana), Whiting, Ind.

The analysis of industrial water for dissolved calcium, magnesium, sodium, and iron by conventional chemical methods is tedious and time-consuming. The Beckman flame photometer with photomultiplier tube offers a rapid and accurate method. The ranges of concentrations of ions determined by this method are: calcium, 30 to 45 p.p.m.; magnesium, 5 to 15 p.p.m.; sodium, 5 to 25 p.p.m.; and iron, 1 to 2 p.p.m. Precision and accuracy for each are about 0.1 p.p.m. No sample preparation is required and the complete analysis for the four ions takes 20 minutes. The effects of fuel type and pressure and the interinfluence of the ions in the sample have been studied.

Use of Radioactive Phosphors as Luminous Standards in Flame Photometry. Determination of Calcium. W. B. Mason, Atomic Energy Project and Department of Biochemistry, School of Medicine and Dentistry, University of Rochester, Rochester, N. Y.

A procedure for determining calcium by flame photometry is described in which the intensity of the calcium emission at 554 $m\mu$ relative to the flame background at 547 $m\mu$ is compared to the intensity of a Sr^{90} activated phosphor. The intensity measurements are made with a Beckman Model DU spectrophotometer modified by substitution of a Farrand 1P21 electron multiplier photometer as detector. Flow of oxygen, propane, and air into the flame is controlled with the aid of individual flowmeters to provide a constant flame. The solution being analyzed contains a standard amount of lithium, and the rate of solution flow into the flame is adjusted to give a specified lithium emission at 670 $m\mu$. A second spectrophotometer is used to measure the lithium emission. The intensity of the calcium emission is a linear function of calcium concentration over the range studied (0 to 24 p.p.m. of calcium) and is essentially independent of sodium, potassium, and phosphate concentrations.

Application of Organic Solvent Extraction to Flame Photometry. John A. Dean and J. Harold Lady, Department of Chemistry, University of Tennessee, Knoxville, Tenn.

Certain advantages are inherent in the process of extracting metal chelates with an organic solvent and then aspirating this organic extract directly into the flame for the determination of the element(s) extracted. This procedure permits separations from large amounts of potential interferences for any given flame photometric method, gives a large increase in emission sensitivity, provides a solution for aspiration whose physical properties are nearly constant, and avoids cooling of the flame through the introduction of large concentrations of diverse elements. Furthermore, the combustible organic extractant also contributes greatly to the size and energy of the flame.

As an illustration of this type of procedure, the analysis for iron is presented. Acetylacetone was employed as the extractant and chelating agent. Optimum operational conditions have been developed using the Beckman Model DU spectrophotometer with flame attachment and photomultiplier unit. By the proposed procedure the determination of iron can be accomplished in the presence of very large amounts of calcium, magnesium, and aluminum. Replicate samples show a standard deviation less than 3%. Sensitivity of iron is 1.0 p.p.m. per instrument scale division. Results are shown for aluminum-base alloys and various types of limestone samples.

Determination of the Origin of Opium by Means of the Composition of the Ash. J. C. Bartlet, C. G. Farmilo, and L. I. Pugsley, Food and Drug Laboratories, Ottawa, Ontario, Canada.

Much of the illicit narcotic trade of the world is concerned with opium. It is not only used as is but is the source of morphine and the indirect source of heroin. In order to control the international drug traffic, the geographical source of illicit opium must be known, so that supplies of the drug may be cut off at the start.

In the past few years, the United Nations Secretariat has acted as coordinator for research aimed at devising chemical and physical means of identifying the origin of opium. As a part of this program, the composition of the ash of over 100 opium samples of known origin has been determined flame photometrically, spectrographically, and chemically. From the data collected it has been possible to reduce criteria for the determination of the geographical origin of unknown samples. These criteria have been tested by the determination of the origin of twenty "unknown" samples. The validity of the method is demonstrated by the fact that each of the twenty unknowns was identified correctly.

Spectral Line Intensities of Iron and of Nickel in Arc Discharges in Inert Gas Atmospheres. Kenneth B. Mitchell (present address, AEC, Los Alamos, N. M.), William J. Nolan (present address, General Electric Co., Schenectady, N. Y.), and Mary E. Warga, University of Pittsburgh, Pittsburgh, Pa.

Spectra of iron and of nickel produced in a 220-volt direct current arc discharge in neon, argon, and krypton atmospheres were studied. Ratios of the relative intensities of a number of lines observed in each of the three atmospheres were calculated. A new stainless steel, gas-tight arc housing was designed for and used in these studies.

Physical Basis of Spectral Line Enhancement in the Noble Gas Direct Current Arc. Milton R. Baker, S. James Adelstein, and Bert L. Vallee, Biophysics Research Laboratory, Department of Medicine, Harvard Medical School, and Peter Bent Brigham Hospital, Boston, Mass.

It has been reported that in argon and helium certain spectral lines are selectively enhanced. The rates of volatilization of elements in direct current arc sources surrounded by these gases are markedly altered from those observed in air. In addition, the over-all background is reduced.

Further studies have confirmed the enhancements of spectral lines previously noticed in argon and helium and have newly demonstrated a similar effect in krypton. The magnitude of the effect is a function of the concentration in which the elements occur in the sample—as has been reported—the enhancement being the greater the lower the concentration. The largest enhancements for a fixed concentration of elements are observed for lines, the excitation potentials of which are in the vicinity of the first excited levels of the noble gas atmosphere employed. It is concluded that a mechanism of excitation in these sources is as follows: The noble gas is energized to its lowest excited levels by the arc discharge. Through collisions of the second kind, this energy is transferred to neutral metal atoms present. The metal atoms are thus ionized and excited. The ion lines of the metal atoms whose excitation potential occurs in the vicinity of the lowest excited levels of the gas employed are consequently strongly enhanced.

The excitation of lines of the neutral atoms, on the other hand, primarily occurs through thermal collisions.

The experimental findings and the physical basis of these phenomena are documented and discussed.

Anode Temperatures in the Noble Gas Direct Current Arc with Alkali and Alkaline Earth Metals. BERT L. VALLEE AND RALPH E. THIERS, Biophysics Research Laboratory, Department of Medicine, Harvard Medical School, and Peter Bent Brigham Hospital, Boston, Mass.

Marked differences in temperature of the electrodes have been noted previously in the direct current arc when operating in helium, neon, and argon, and data have now been obtained in krypton. With any particular gas high reproducibility of temperature conditions was exhibited. Marked differences in the voltage drop across the arc were also observed in these gases. These measurements were performed on pure graphite rods. A recording pyrometer constructed in this laboratory was employed.

Using this system the studies have now been extended to measurements of anode temperatures when the anode contains a sample. The chlorides of the elements from groups I and II of the periodic system were arced individually. No mixtures of these salts have been studied thus far. Temperature measurements were performed in helium and argon. Simultaneously, the voltage across the gap was recorded.

Preliminary analyses of the data indicate a correlation of the temperature to the boiling points of the salts and the position of the cation in the periodic table. Similarly, there was good correlation between the temperatures observed and the disappearance of the salt from the electrode. The presence of all salts depresses the voltage, more in column IA than in column IIA in a given gas, but the magnitude of the depression differs between helium and argon.

The implications and experimental detail are discussed.

Infrared Intensities of Simple Molecules. BRYCE CRAWFORD, JR., University of Minnesota, Minneapolis, Minn.

Modern infrared instrumentation and the development of appropriate techniques have made possible fairly accurate measurements of vibrational intensities (integrated band intensities) for simple gas-phase molecules. These data tell us how much the dipole moment of the molecule changes with the normal vibration being studied: the band intensity is proportional to the square of the derivative dp/dQ, p being the moment and Q the normal coordinate. For diatomic molecules, this derivative is an "effective charge"; the available data on diatomics are reviewed.

The question of the sign of the derivative is difficult to settle, no general method being available. This point is interesting even for diatomics and becomes a major problem in considering polyatomic molecules. The known possible ways of determining some of the signs (through high-resolution studies of rotation-vibration interaction; through comparison of isotopic molecules; through consideration of the bond-moment approximation) are discussed and cases shown of their applicability.

The ultimate interest of such data lies in their interpretation in terms of the charge distribution in molecules. The crudest picture, that of effective charges firmly attached to the several atoms, is shown to be hopelessly inaccurate. The next simplest assumption, that bond moments may be attached to the several bonds, seems to be a useful qualitative approach which, however, has no quantitative validity. The data are presented on which these conclusions rest, and possible more sophisticated interpretations are discussed.

Infrared Intensity of the S—H Stretching Vibration. FRANKLIN BYERS AND ROBERT A. SPURR, University of Maryland, College Park, Md.

Infrared intensity measurements of the S—H stretching band at 2580 cm.$^{-1}$ give evidence of hydrogen-bonding of mercaptans in carbon tetrachloride solution. On dimer formation the band is shifted very slightly to lower frequencies. There is a considerable enhancement of intensity. From the variation of the intensity of the unresolved band with frequency the dissociation constant of the dimer can be deduced.

Quantitative Study of the Bonding of Chloroform-*d* in Various Solvents by Infrared Spectrometry. R. C. LORD, B. NOLIN (present address, Carothers Research Laboratory, Experimental Station, E. I. du Pont de Nemours & Co., Wilmington 98, Del.), AND H. D. STIDHAM, Spectroscopy Laboratory and Department of Chemistry, Massachusetts Institute of Technology, Cambridge 39, Mass.

Infrared studies have shown that the intensity of the carbon-deuterium stretching band in chloroform-*d* increases tenfold or more when the latter is mixed with certain polar solvents. This increase has been measured quantitatively for diethyl and di-*n*-butyl ethers, *p*-dioxane, anisole, acetone, acetic anhydride, ethyl acetate, and pyridine. The association constant for the system diethyl ether-chloroform-*d* has been found to be 0.80 ± 0.15 liter per mole from the infrared data.

Automatic Integrator for Measuring the Intensities of Infrared Absorption Bands. VINCENT J. COATES AND FRANK GAARDE, The Perkin-Elmer Corp., Norwalk, Conn.

The functional groups of organic molecules have generally been characterized by the wave lengths or frequencies at which they absorb infrared radiation. Little attention has been paid to the intensity of radiation absorbed by the group as a function of its molecular environment. Recent studies have indicated that absolute intensity measurement may yield information which can provide a new dimension for structural analysis. There exists also the possibility that measurement of the total intensity may improve quantitative accuracy.

One reason for the general lack of enthusiasm for pursuing such studies has been the difficulty of obtaining precise total intensity measurements. This paper discusses an automatic, absolute intensity integrator attachment to the Perkin-Elmer 21 linear transmittance recording infrared spectrometer. This device provides accurate and continuous integration of the function,

$$A_0 = \frac{K}{Cl}\int_{v_1}^{v_2} \log_{10}\frac{1}{T}\,dv$$

where A_0 is the absolute intensity, K is a constant which depends on the instrument scale factors, v_1 to v_2 is the frequency interval of integration.

A mechanical cam converts the transmittance output of the spectrometer to linear absorbance over the range 0 to 1.0. As the spectral interval is scanned, the wave length or frequency and absorbance are fed into a precision ball and disk integrator. The output is read out on a high-speed magnetic counter which also sends impulses to an auxiliary pen system which marks the recording paper on the margin every ten integrated units. The performance of the integrator attachment is shown.

Statistical Study of the Wyandotte-ASTM Punched Card Library of Infrared Absorption Spectra. MARSHALL V. OTIS, JULIAN CHAUDET, WILBUR KAYE, THOMAS R. BIDDISON, AND RALPH ROSE, Tennessee Eastman Co., Division of Eastman Kodak Co., Kingsport, Tenn.

The library of 7490 IBM cards for organic compounds coded under the auspices of ASTM Committee E-13 was used in a statistical study to correlate code punches for chemical classification, absorption bands, and general structure. The study was based on the frequency distribution of punches for 12 general structure codes, 15 functional group codes, and absorption bands in the skeletal region of 10 to 14 microns.

Frequency distribution histograms were plotted for the various codes. Distribution of strong band assignment for —C═N absorption made a special sorting sequence necessary. Absorption band punches for the 8.0- to 8.9-micron region were tabulated. The tabulation showed that the high incidence of punches did not aid the sorting resolution for the 15 functional group codes. By use of an IBM Model 402 tabulator, other spectra-structure correlations may be made. A typical application of the identification of the compounds in a three-component system is described in detail.

A rapid separation of cards which are significant in solving a particular problem may be made by a single pass of the library of cards through an IBM Type 101 electronic statistical machine.

Analytical Applications of Far Infrared Spectra. F. F. BENTLEY, N. E. SRP, G. D. OSHESKY, AND W. R. POWELL, Materials Laboratory, Wright Air Development Center, Dayton, Ohio.

Spectra obtained in the 15- to 35-micron region are particularly useful in material characterization. Slight changes in structure produce considerable changes in spectra, giving a more specific "fingerprint." Furthermore, use of the far infrared for group identification is not excluded. Frequencies characteristic of bromine, iodine, sulfur, and other derivatives of heavy molecules are observed in this region. In several instances far infrared gave solutions to analytical problems for which the 2- to 16-micron region did not.

Conventional infrared techniques are used, and in some instances simplified, in obtaining far infrared spectra. Many common solvents such as carbon disulfide, dimethyl sulfide, thiophene, furan, benzene, toluene, etc., have large "open windows." The less polar solvents can be used in cells as thick as 2 mm. while solvents as polar as acetone can be used in 0.1-mm. cells. Nujol has no significant absorption bands in this region. The spectra of greases, high polymers, etc., can be obtained by using strips of polyethylene to support the materials, thereby eliminating the use of costly cesium bromide plates.

The use of such infrared spectra in the qualitative and quantitative analysis of aircraft materials is discussed.

Analytical Applications in the Near Infrared. HENRY J. NOEBELS, Beckman Instruments Corp., Fullerton, Calif.

Until recently the spectral region from 1 to 2.8 microns has not been generally available to analytical chemists. Consequently, very few articles concerning analyses in this region have appeared in the literature. This region is attractive for analytical work, since recent developments in instrumentation allow the user the convenience of the technique normally used on the ultraviolet and visible, plus some of the specificity of the infrared region.

Applications of this region to a number of analytical problems commonly encountered are given. In addition, a number of near infrared curves of compounds of interest are shown.

Decrease with Time of Sulfuric Acid-Induced Fluorescence of Steroids. HERBERT S. STRICKLER, ROBERT C. GRAUER, AND MARY RUTH CAUGHEY, Singer Memorial Laboratory, Allegheny General Hospital, Pittsburgh, Pa.

Measurement of the fluorescence of estrone, estradiol, and estriol at various time intervals after sensitizing by heating with sulfuric acid reveals that the fluorescence falls off according to a definite law. The differential equation which applies is $dG/dt = -AG$, where G is the galvanometer reading corrected for impurities, t is the time, and A is a constant for a given estrogen and specimen. In this formulation, the assumption is made that the impurity contribution is a constant. This assumption is valid for conventional urinary extracts after countercurrent distribution or use of chromatography. The application to other steroids and other types of compounds, and to other biological extracts is currently being explored. The derivation of convenient equations and supporting data is presented.

Automatic Spectrophotometric Titration with Coulometrically Generated Titanous Ion. Determination of Vanadium in Titanium Tetrachloride. H. V. MALMSTADT AND C. B. ROBERTS, Noyes Chemistry Laboratories, University of Illinois, Urbana, Ill.

Titanium tetrachloride is prepared for analysis by pipetting a sample into a solution of hydrochloric acid in a flask fitted with a reflux condenser and water trap. The vanadium in the hydrolyzed sample is then determined directly by a rapid and accurate titrimetric procedure using automatic spectrophotometric end-point detection and coulometric generation of titanous ion. The amount of vanadium is determined by the quantity of titanous ion required to go from the first end point (V^{+++++} to V^{++++}) to the second end point (V^{++++}, V^{+++}). Ten milliliter samples containing about 0.01 to 0.2% vanadium are determined with an average deviation of about 0.00016%.

A Spark-in-Spray Excitation Method for the Emission Spectrochemical Analysis of Vanadium and Iron in Titanium Tetrachloride. H. V. MALMSTADT AND R. G. SCHOLZ, Noyes Chemistry Laboratories, University of Illinois, Urbana, Ill.

A solution excitation technique for emission spectroscopy is described which is rapid, inexpensive, accurate, and directly applicable for the determination of vanadium and iron in hydrolyzed titanium tetrachloride solutions. The solution is sprayed from an atomizer between two horizontal graphite electrodes across which a controlled high voltage spark is applied, and is referred to as the spark-in-spray method. Source conditions were studied and working curves for vanadium and iron in titanium tetrachloride were plotted for the concentration ranges of 0.003 to 0.2% and 0.005 to 0.1%, respectively. Relative average deviations of 1.2% for vanadium and 4.8% for iron were obtained.

Polarographic and Spectrographic Analysis of Silicon-Germanium Alloys. MARVIN C. GARDELS, Radio Corp. of America, RCA Research Laboratories, Princeton, N. J.

During a study of germanium-silicon alloys for possible transistor applications, it was necessary to analyze the alloys quantitatively to correlate certain electrical properties and composition. A polarographic method which required no chemical separation was developed for the determination of germanium in the presence of silicon. The alloys were dissolved by fusing them with sodium hydroxide, dissolving the melt in water, and boiling with hydrogen peroxide to complete the solution. Aliquot portions were removed and polarographed in a carrier electrolyte of $0.1N$ sodium chloride adjusted to a pH of 10.2. A dropping mercury cathode and a mercury pool anode were used. The half-wave potential of germanium was -1.54 volts *vs.* the mercury pool. The diffusion currents were read at -1.8 volts for a germanium concentration of from 10 to 40 mg. of germanium per liter of solution.

To obtain more accurate analyses for alloys containing less than 15 mole % silicon, a spectrographic technique for silicon was developed. To burn a 10-mg. sample completely, it required 2 minutes in a 10-ampere direct current arc. The exposure was controlled with a rotating sector. With Spectrum Analysis No. 1 plates, the line pair Si 2435/Ge 2436 was found suitable for a concentration of from 1 to 5 mole % silicon, and the pair Si 2987/Ge 3067 from 5 to 15 mole % silicon. Suitable line pairs for intermediate concentrations of from 2.5 to 10 mole % silicon were Si 2631/Ge 2644 and Si 2631/Ge 2556. The line pair was chosen according to the amount of silicon present.

Quantitative Spectrographic Analysis for Trace Impurities in Silicon. JOSEPH S. RUDOLPH AND MARY LOUISE BACON, Westinghouse Electric Corp., East Pittsburgh, Pa.

A rapid and sensitive spectrochemical method for the simultaneous determination of eighteen elements in silicon metal is described. Au, Mn, Fe, Mg, Cu, Cr, Sn, Ga, Ni, Ca, Ti, Tl, Pb, Bi, Li, and In can be determined to 10^{-5} weight %; Zn and Sb to 10^{-4} weight %. The average "standard deviation" for these determinations is approximately ±20%.

In order to achieve the required sensitivities for close quality control of semiconductor grade silicon, the impurities are concentrated by digesting 10 grams of sample in a solution of nitric and hydrofluoric acid with subsequent volatilization of the silicon as a fluoride. The resulting solution is evaporated to dryness after adding graphite as a carrier, cadmium as an internal standard, and sulfuric acid to convert the cations to sulfates. Thoroughly mixed aliquots are excited in a direct current arc.

Chemical Analysis of the NBS-GMC Zinc-Base Standards for Manganese, Nickel, and Chromium. R. E. KOHN AND M. D. COOPER, Research Laboratories Division, General Motors Corp., Detroit, Mich.

Methods have been developed for evaluating the homogeneity and certification of zinc-base spectrographic standards with respect to manganese, nickel, and chromium at levels below 0.05%. Familiar photometric methods for the determination of manganese and nickel in other alloys have been found to apply. Chromium is determined by a photometric method in which diphenylcarbazide reacts with chromium oxidized to the hexavalent state by means of potassium permanganate.

Homogeneity and precision data are presented.

Homogeneity Study of NBS-GMC Zinc-Base Die-Cast Alloy Standards. R. C. FRANK AND J. E. DALLEMAND, Research Laboratories Division, General Motors Corp., Detroit, Mich.

Five samples, $1^3/_4 \times 1^3/_4 \times {}^1/_2$ inch, chosen carefully from each of six 3000-pound heats were analyzed at ten prescribed positions on their surfaces with a direct reading spectrograph. Each analysis was made for the following eleven elements: Al, Cu, Mg, Pb, Sn, Cd, Fe, Cr, Ni, Mn, and Si. The data were processed using an analysis of variance procedure on an IBM card program calculator. Three variables were considered: surface location, sample location in the heat, and time effects in the spectrographic method. The Duncan test was used to determine which surface locations were significantly different from others, the Cochran test was used to determine if any surface location gave less reproducibility than others, and the data were checked for nonsymmetry of extreme values.

This paper briefly describes the statistical procedures used and emphasizes the evaluation of the statistical results in terms of the practical situation.

A Combined Chemical-Spectrographic Method for the Quantitative Determination of Microgram Amounts of Certain Rare Earths in Zirconium Metal. H. HETTEL AND V. A. FASSEL, Institute for Atomic Research and Department of Chemistry, Iowa State College, Ames, Iowa.

The presence of fractional part per million quantities of several of the rare earth elements in zirconium metal used as structural material in thermal reactors can reduce the regenerative efficiency and neutron economy of these reactors. To determine the rare earth content at such a low concentration level, a combined chemical concentration-spectrographic determination method has been developed. This method permits the quantitative determination of gadolinium, samarium, dysprosium, holmium, and terbium in zirconium metal in the concentration range 0.05 to 1 p.p.m. One hundred-gram samples of zirconium metal sample are dissolved in hydrofluoric acid and after diluting the resulting solution to 2000 ml., 20 mg. of yttrium carrier are added. Yttrium fluoride is not precipitated under the environmental conditions of this solution. The solution is then passed through a cation exchange resin. The ZrF_6^{--} anions pass through the column while the rare earth and other cations undergo ion exchange and are retained on the column. Subsequent elution of the column with hydrochloric acid yields a solution which contains only a trace of zirconium. The yttrium and other rare earths are separated from the other cations which may be present in the sample by standard chemical procedures. The rare earth elements are then determined in the yttrium matrix by standard spectrographic procedures. Data are presented showing that quantitative recoveries are obtained.

The Quantograph, a Combination Direct-Reading Spectrometer, Monochromator, and Spectrograph. E. Davidson, J. L. Jones, and P. S. Goodwin, Applied Research Laboratories, Glendale, Calif.

A Czerny-Turner combination spectrometer, monochromator, and spectrograph has been developed which allows a single instrument to be utilized as three separate instruments, each instantly available without any external change in the unit.

The Quantograph can be supplied with any one, a combination of any two, or all three of the basic units to meet the varied spectrochemical needs of analytical laboratories.

The spectrometer allows the instrument to be set up for high-speed, direct-reading analysis with a permanent array of receiver slits and phototubes.

The monochromator allows its use for the direct-reading analysis of any element—the spectrum of which can be excited—in any matrix, thus making it a universal direct reader.

The spectrograph allows its use in a conventional manner as a wide-range spectrograph possessing excellent dispersion and resolution.

The unit has been designed to mount on the new, unitized source described in another paper and employs the small, basic recording console of the quantometer when used as a direct-reading instrument.

Direct-Reading Attachment for a Medium Quartz Spectrograph. A. C. Menzies, Hilger & Watts, Ltd., London, England.

A description is given of an attachment which replaces the photographic plate in a medium quartz spectrograph, and typical results of analyses are stated.

The attachment contains eleven multipliers, with slits in the focal plane of the spectrograph which can be moved to select eleven spectral lines. A novel form of compensation for temperature effects is provided.

The multiplier currents are integrated, and the exposure can be regulated either by time control, or by the potential of any particular condenser reaching a preset value. After starting the source, all the operations proceed automatically.

Switches are provided which allow the measurement of the potential of any condenser to be omitted, so that time need not be wasted on measurements which are not required.

The reproducibility of the equipment for steady intensity ratios is 0.25%. So far, it has been used for aluminum, magnesium, and lead-based alloys, and for the determination of additives in lubricating oil.

Direct-Reading Spectrometer for Foundry Floor Operation. J. L. Saunderson, E. DuBois, and J. H. Jurman, Baird Associates, Inc., Cambridge, Mass.

A new and smaller direct-reading spectrometer, the Spectromette, making possible analytical control of melts directly on the foundry floor, is described. Important new features include completely sealed optical and electronic compartments and an automatic monitor system which continuously maintains proper optical alignment. Simplified controls and electronic circuits designed conservatively using long-life "reliable" tubes as well as interchangeability of units assure a minimum of "down-time." This instrument combines rapid analytical control with low installation and operational cost.

Theory and Design of Spectrocomputers for Emission Analysis. John S. Wolfe, Delco Products, Division of General Motors Corp., Dayton, Ohio.

This paper relates to the theory and construction of spectrographic computers and computing circuits for direct readers. The first portions, applicable only to photographic installations, describe a method for correcting errors arising from comparing intensities of lines having different gammas, affording a means of valid computation with lines having any wave-length separation; a modified computing procedure including this principle consolidates the separate calibrations for gamma and for curve shift into a single standardization operation.

The second portion describes a phenomenon observed in arc and spark discharges which permits prediction of spectrum line intensities, and the computative use of this principle. Artificial homologs are computed and compared with element lines of any excitation potential. The method permits a much wider choice of analytical lines and affords compensation for minor excitation variables, and its application to both photographic and direct reading instruments is discussed. No additional operation is involved.

Computers embodying these concepts have an additional peculiarity in that their working curves are completely general, since a working curve for a specific line may be used unchanged in any laboratory, under a wide range of excitation conditions, and with only minor predictable variation for changes in matrix.

Operation of New Direct Reader for Raw Alloy Steels. F. McNally, Jarrel-Ash Co., Newtonville, Mass.

The information supplied by the present JACO direct reader includes slag V-ratio Si, Cu, Ni, Cr, Mo, and Sn.

A sample preparation technique has been worked out for slag which makes possible a $2^1/_2$-minute analysis. Reproducibility and long term stability are superior to the previously used photographic method. The use of relatively weak silicon and calcium lines has made this possible since the usually strong lines are nonsymmetrical in nature.

The ability and reproducibility data as well as other work are described.

Quantometric Analyses of Titanium Metals. E. M. DuBois and Arno Tuteur, Spectrochemical Laboratories, Inc., Pittsburgh, Pa.

A vapor injection technique is presented for the quantometric analysis of titanium metals. In addition, the point to plane procedure is described, which can be used for studying segregation in titanium alloys. A multiple point to plane method is also introduced which will give reproducible results on solid samples of titanium which show segregation. These investigations were based on a series of samples supplied by Wright-Patterson Air Force Base, Dayton, Ohio, under a research program sponsored by the U. S. Air Force.

Fisher Spectro Analyzer. A. M. Laing, Fisher Scientific Co., Pittsburgh, Pa.

The Fisher Spectro Analyzer is an electronic spectrograph capable of rapid, precise determination of the constituent elements in as many as five different matrices. The over-all design and principles employed have been derived from 9 years of development and experience. The optical system is a unique departure from the conventional grating mounts. Utilizing two spectra with a dispersion of 3 A. per mm. permits the use of up to 65 individual pickups in the region of 2000 to 6000 A. first order.

The use of individual, plug-in type, alternating current amplifiers embodies the ultimate in electronic stability. This system permits the use of electronic control of the working curves without the use of attenuators, complex relay systems, or mechanical corrections. Alternating current amplification totally ignores the dark current component of the photomultiplier, making for a more favorable concentration to noise ratio. Integration is accomplished electronically and presented on digital counters.

An electronically controlled source is an integral part of the instrument and discharge circuit parameters may be varied to meet various analytical demands. The use of an all-electronic source has been found to have excellent stability and little or no maintenance.

Contrast Changes of Eastman Kodak Spectrum Analysis No. 1 Emulsion during Storage and Their Effect on Observed Intensity Ratios. Cyrus Feldman and Janus Y. Ellenburg, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Film stored one year at 40° F. (4.4° C.) showed an increase in Seidel calibration curve slope ranging from 5 to 12% at various wave lengths. Film from the same lot, stored one year at 0° F. (−17.8° C.) showed decreases ranging from 0 to 6%. Tables have been constructed to show the degree to which a given line-to-line intensity ratio would be distorted (at various line-to-background ratios) by undetected changes in emulsion contrast.

Conditions in Densitometry. Ralph E. Thiers, Biophysics Research Laboratory, Department of Medicine, Harvard Medical School, and Peter Bent Brigham Hospital, Boston, Mass.

In the course of investigations in quantitative spectrochemical trace analysis erratic precisions and accuracies were noticed when 103-0 plates were employed. The use of this emulsion, deemed mandatory by the low concentrational content of the samples analyzed led to investigations—among other factors—of the densitometric methods used. Three different densitometers—two of them of the same make—were found highly individualistic in their performance on these background-rich plates. Data obtained with them were interconvertible only when the calibration curves allowing for the distinctive features of each instrument were constructed. While this aided the analytical performance it was not deemed satisfactory until plates were developed to constant gamma. This required a development time of 12 instead of 3 minutes as recommended by the manufacturer.

The characteristics of this emulsion, seemingly indispensable for the purpose at hand, imply that further changes in the evaluation of photometric data may become necessary.

A Rotating Step-Sector for Use with Alternating Current or Other Intermittent Spectrographic Light Sources. Richard N. Kniseley and Velmer A. Fassel, Institute for Atomic Research, and Department of Chemistry, Iowa State College, Ames, Iowa.

The calibration of photographic emulsions using rotating step-sectors and intermittent spectrographic light sources may lead to erroneous results because of stroboscopic or synchronization effects. Theirs has demonstrated that a focal plane step-sector with randomly spaced apertures can eliminate errors arising from these effects, except under very extreme conditions. The present investigation has dealt with the development of a rotating step-sector which eliminates these effects and which can be readily interchanged with a standard rotating step-sector employed at the spectrograph slit. This sector essentially consists of a series of stepped apertures randomly spaced about the periphery of a rotating disk. Data are presented to show that the random spacing of the sector apertures eliminates the possibilities of introducing errors through synchronization effects when conventional intermittent discharges are used.

Jarrell-Ash Comparator-Microphotometer. HAROLD BEAL, R. F. JARRELL, AND R. K. BREHM, Jarrell-Ash Co., Newtonville, Mass.

The Model 2100 Console comparator-microphotometer recently introduced by the Jarrell-Ash Co. is discussed. The outstanding features of the instrument are:

1. A large view screen, $10\frac{3}{4} \times 10\frac{3}{4}$ inches, of high intensity with a magnification of 15×.
2. Equal illumination of the reference and unknown spectra permitting visual estimation of relative densities of analytical and standard lines.
3. All controls, including those for lateral and transverse plate motion, grouped on the front of the instrument and work space for tabulating results.
4. Extremely high sensitivity. Full scale deflection can be obtained with an aperture equivalent to 500 square microns at the plate.
5. A high speed, rugged detection system not affected by vibration or voltage fluctuations. No voltage regulation is required to give long and short term stability of ±0.5%. The conventional galvanometer or meter is eliminated completely and a Bristol indicating drum potentiometer with a moving transmission scale reading directly to 0.2% *T* projected on the front panel is substituted.
6. Over-all reproducibility to better than ±0.5%.

Performance data including stability, reproducibility, linearity, per cent scattered light and resolution are also given.

A Unitized Optical Emission Source Unit. W. E. FOWLER, P. S. GOODWIN, AND M. F. HASLER, Applied Research Laboratories, Glendale, Calif.

A new, unitized source unit has been developed for use in optical emission spectrochemistry, which combines in a new form the time-tested types of sources pioneered by our laboratories.

The source has been so unitized that any desired type of source can be supplied at the outset and then added to as the need for other sources arises.

The design utilizes the high voltage engineering approach, wherein all high voltage components, including switches, are mounted within an oil-filled, water-cooled case. This eliminates all losses due to corona and leakage and provides the optimum in source stability.

After considerable study of high voltage thyratron ignition, the superiority of rotary gap control was again demonstrated and thus has been retained in the new source. However, the gap is of an entirely new design, which should minimize maintenance and provide long-term, trouble-free service.

The use of oil immersion for all high voltage sections has allowed a very compact design. Thus, the source cabinet is of table height and can be used as the base for the Quantograph described in another paper. This design allows the use of very short cables between the source and the arc-spark stands of the various spectrographs and Quantometers with which the source will be used.

New Vacuum Spectrographs. A. C. MENZIES, Hilger & Watts, Ltd., London, England.

Vacuum spectrographs, using gratings and prisms have been made in England for many years. Some of these have now been redesigned, and new instruments added to the range.

A brief description is given of normal incidence and grazing incidence vacuum spectrographs, and a new three-prism fluorite spectrograph designed for direct reading and for use with photographic plates, with provision also for visual observation of strong lines.

In designing the instrument last referred to, the problems of analysis of phosphorus and sulfur have been particularly in mind.

Improvement of Analytical Sensitivity by Increased Spectrographic Dispersion. R. K. BREHM AND R. F. JARRELL, Jarrell-Ash Co., Newtonville, Mass.

For years it has been assumed that the lower limit of detectability in spectrochemical analysis can be improved by employing greater and greater dispersion. Although a number of empirical attempts to prove this theory have failed, the basic idea persists. This paper presents data to demonstrate the effects of increasing dispersion on the lower limit of detectability for a number of materials and discusses the conclusions to be drawn. The concepts of "optimum" and "empty" dispersion are introduced.

High Resolution Work with the Baird Associates Modified Eagle Mount Spectrograph. R. O'B. CARPENTER AND L. O. EIKREM, Baird Associates, Inc., Cambridge, Mass.

Experiments have been conducted to determine the efficiency of the higher orders of the Baird Associates modified Eagle Mount spectograph for high resolution work. A discussion of the expected aberrations in the Eagle mount will demonstrate that this mount provides for excellent results in higher orders, and these prognostications have been confirmed by experiment. Experiments have been conducted using the new Baird Associates 20-inch camera instrument. Resolutions over 100,000 have been obtained in Order III, and excellent line definition has been maintained in Order VII with a 15,000 line per inch grating. A Baird Associates microwave excited mercury 198 source of line width 0.005 A. at 5461 A. has been used in some of the evaluation work.

Results obtained using a crossed Fabry-Perot Etalon at Sirks' secondary focal position of the Eagle Mount spectrography are also shown. Multilayer dielectric reflectors were used to produce higher resolution and optical speed (above 90% transmission) than possible to attain with silver with aluminum coatings. Resolutions of several million have been obtained with this arrangement. Experiments confirm the theory of Pierre Jacquinot on the relationship of optical speed to resolution. The Fabry-Perot Etalon and condensing lens are mounted on a single optical bench rider for convenient setting at the secondary focal position.

Analytical Application of the Ebert Spectrograph with Order Sorter. R. K. BREHM AND R. F. JARRELL, Jarrell-Ash Co., Newtonville, Mass.

The Ebert spectrograph manufactured by the Jarrell-Ash Co., has been in use by many laboratories for more than one year. Up to this time the instrument has been used in a conventional manner. Now data are presented giving typical results obtained with the Ebert spectrograph in combination with the Order Sorter, showing the increased range of application to difficult analytical problems where the matter of dispersion is important. These include problems where interferences make it impossible to use the more sensitive lines or where the lines themselves are buried in the cyanogen bands. Working curves of typical analysis, precision, sensitivity, and wave-length coverage are given.

Testing the Performance of a Spectrophotometer. HOWARD CARY AND ROLAND C. HAWES, Applied Physics Corp., Pasadena, Calif.

Departures from ideal performance in a spectrophotometer can lead to embarrassing discrepancies in qualitative and quantitative analysis. Some details of instrument performance leading to such discrepancies are discussed. Tests for evaluating instrument performance in respect to wave-length calibration, resolving power, remote and near scattered light, photometric accuracy, signal-noise ratio, and sample beam collimation are presented and illustrated by results on an instrument covering the wave-length range 0.19 to 2.6 microns.

Design and Performance of a Direct Ratio Automatic Recording Universal Spectrophotometer. V. Z. WILLIAMS AND C. D. COWLES, Perkin-Elmer Corp., Norwalk, Conn.

The design of an unusually versatile spectrophotometer is discussed. The instrument provides continuous double-beam ratio recording with optimum performance in the ultraviolet, visible, and infrared regions, with various sources, prisms, and detectors. Provision is made for convenient interchange of these components. The slits are automatically controlled for constant energy with any source-prism-detector combination. In addition, the instrument may be converted easily to single-beam operation, the slits being programmed by the slit servo. This flexibility permits use of building block accessories such as the infrared microscope. Performance data and spectra are presented in the various regions from 0.2 to 38 microns.

Scanalyzer. Automatic Ultraviolet Spectral Scanning of Chromatographic Effluents. W. C. KENYON, J. E. MCCARLEY, E. G. BOUCHER, A. E. ROBINSON, AND A. K. WIEBE, Hercules Experiment Station, Hercules Powder Co., Wilmington, Del.

The analysis of complex mixtures of aromatic and other compounds by ultraviolet spectrophotometry is hindered by overlapping spectra. Chromatography is an excellent technique for separating dilute solutions of complex mixtures. It is, therefore, an ideal supplement to ultraviolet spectrophotometry, which is sensitive enough to detect the low concentrations present in the effluent. However, the hand collection of effluent cuts and subsequent determination of their ultraviolet absorption spectra are time-consuming and costly.

This paper describes an attachment to a Cary spectrophotometer with which the ultraviolet absorption spectrum can be obtained automatically on each of 200 or more chromatographic cuts. Thus, one obtains a complete record of the ultraviolet absorption spectra of each compound present in a mixture. This record is displayed on one chart after a 16-hour overnight run. No attention from an operator is required after the start. From this chart, qualitative identification and quantitative determination of the compounds in the mixture can be made.

In order to make the technique applicable to wide varieties of compounds, the eluent is gradually increased in polarity during a run—(gradient elution). The fractions scanned are selected on a volume, rather than time, basis, since precise quantitative data can be more easily obtained by this means. Thus, one avoids having to solve the very difficult problem of maintaining the very small flow rate exactly constant.

With this apparatus, as many as twenty different compounds have been detected in one sample. A quantitative analysis is presented of a synthetic mixture of eight aromatic compounds. The results agree within ±3% of the amounts present.

The principles of this device can also be applied to infrared spectral scanning, although the lack of suitably transparent solvents reduces the versatility of the method.

A New Spectrophotometer for Use with Volumes of 0.020 Ml. FREDERICK BRECH, Jarrell Ash Co., Newtonville, Mass., AND DAVID GLICK, Department of Physiological Chemistry, University of Minnesota, Minneapolis, Minn.

A double-beam instrument is described for use in the ultraviolet and visible regions between approximately 210 and 600 mμ. A grating monochromator with a reciprocal dispersion of 4.4 mμ per mm. provides two beams, each focused on a separate exit aperture. One beam passes through a cuvette containing the sample and the other passes through a reference cell. A rotating alternator permits each beam in turn to fall on the same area of a single photomultiplier, the output of which is coupled to a logarithmic amplifier. This provides a signal of the form log (A/B) which is displayed on a meter graduated linearly with absorbance. A feature of the instrument is that it employs special cuvettes with a 1-cm. light path having volumes of 0.020-ml. Microcuvettes of this type with light paths up to 5 cm. can be accommodated. Macrocuvettes with light paths from 1 to 5 cm. having volumes of several milliliters can also be used. Examples of the use of the instrument in biochemical and histochemical research are detailed.

New System for Data Presentation in the Beckman DK Spectrophotometer. ALAN BUNKER AND B. D. HENDERSON, Beckman Division, Beckman Instruments, Inc., Fullerton, Calif.

A new system has been developed for data presentation in the Model DK-spectrophotometer discussed last year. A major feature of the new system is an integral recorder and monochromator. Recording is done on 11 × 17 printed charts on a flat sloping platen with completely visible spectra. Wave-length presentation is linear in either wave-length or wave number. A switch provides either absorbance or transmittance with expanded ranges and special ranges for differential recording. The relative merits of printed chart versus strip chart recorders are discussed.

Extended Wave-Length Regions for Beckman Prism Spectrophotometers. J. G. MYERS, Beckman Division, Beckman Instruments, Inc., Fullerton, Calif.

Using either natural crystalline quartz or fused silica prisms, a Beckman Model DU spectrophotometer has been operated in regions considerably lower in the ultraviolet and higher in the infrared than normal. With proper components and detectors, spectral regions have been observed and identified from 185 to 3.5 μ. Stray light values in various regions are given. Traces from a Beckman DK recording spectrophotometer are included.

Applications of the Sum Rule to Vibrational Spectra and Heat Capacities of Homologs. H. J. BERNSTEIN, National Research Council, Ottawa, Canada.

The sum of the vibrational frequencies in halogenated methanes, ethanes, and ethylenes is shown to be an additive property. These sums may be predicted on the basis of an additivity scheme which includes interaction between bonds and/or nonbonded atoms. Application of the sum rule reveals errors in assignments in the literature and predicts sums for molecules hitherto not investigated spectroscopically. A logical development of the sum rule indicates that the heat capacity also is an additive property. Heat capacities of some methanes and ethylenes have been predicted and the temperature dependence of the heat capacity has been shown to be of the form $C_p = A + B/T + C/T^2$ for molecules possessing relatively few hydrogen atoms.

A Comparison of the Deformation Vibration Frequencies of the Methyl Group With Those of the Coordinated Ammonia Molecule. S. MIZUSHIMA, G. F. SVATOS, J. V. QUAGLIANO (present address, Office of Naval Research, Chicago, Ill.), AND BROTHER COLUMBA CURRAN, Department of Chemistry, University of Notre Dame, Notre Dame, Ind.

The deformation vibrations of the methyl group, degenerate, symmetric and rocking, have been characterized by the calculations of Nakagawa and Mizushima. The coordinated ammonia molecule is expected to undergo similar deformation vibrations, and the absorption spectra obtained for several metal ammine complexes reveal this similarity.

The band attributed to the degenerate vibration of the coordinated ammonia molecule is at about the same frequency as in the spectrum of ammonia, appreciably higher than the corresponding methyl group vibration. The symmetric vibration is in the same frequency range as that of the methyl group, higher than that of the free ammonia molecule.

The rocking vibration of the coordinated ammonia molecule, absent in free ammonia, appears to be most directly related to the strength of the nitrogen-to-metal bond. One might expect the band attributed to this vibration to be missing or very weak in the event of a purely ion-dipole nitrogen-to-metal bond. The observed frequency increases sharply with increasing covalent character of this bond, varying from 655 cm.$^{-1}$ in hexammine cobalt(II) chloride to 830 cm.$^{-1}$ in hexammine cobalt(III) chloride.

Quantitative Infrared Analysis of Alkyl Phenol Mixtures. F. V. FAIR AND R. J. FRIEDRICH, Research and Development Division, Pittsburgh Consolidation Coal Co., Library, Pa.

In a research program involving tar from low-temperature carbonization of bituminous coal, the distribution of the phenolic compounds was desirable. Phenol, the three cresols, the six xylenols, *m*-ethylphenol, *p*-ethylphenol, and several of the trimethylphenols can be determined by the described method.

The tar acids are extracted from the tar with sodium hydroxide and then sprung from the aqueous solution with sulfuric acid. After drying, the crude material is fractionally distilled and the cuts are submitted for infrared analysis.

The problems of interferences, distillation cut points, and selection of analytical wave lengths, as well as a solution to these problems are given. The precision of the analysis is 1% absolute and an analysis, starting with the whole tar, can be completed in 24 hours.

A Spectroscopic Study of Fractions of Oil Used in Oil-Rubber Masterbatches. FREDERIC J. LINNIG AND JAMES E. STEWART, National Bureau of Standards, Washington, D. C.

Oils which are by-products of petroleum refining are used as extenders in GR-S synthetic rubber. The properties of the rubber depend to a large extent on the type of oil present. The infrared spectra of five fractions of each of seven oils separated chromatographically were examined. In some cases differences from oil to oil in the intensity of absorption bands at one wave length could be related to differences at other wave lengths and to differences in physical properties such as refractive index. In these cases the absorption bands could thus be related to structure with greater certainty than would otherwise be possible with mixtures of this type. Certain of the fractions obtained by an acid extraction procedure were similar to those obtained chromatographically. Furthermore, there is a similarity between the infrared spectra of some of these oil fractions and certain constituents of asphalt and coal. Ultraviolet spectra in the region above 200 mμ were also obtained.

Infrared Studies of Coordination Compounds. Structural Isomerism of Nitro- and Nitrito- Pentamminecobalt(III) Chlorides. RAY PENLAND, THOMAS J. LANE, C.S.C., AND J. V. QUAGLIANO (present address, Office of Naval Research, Chicago, Ill.), Department of Chemistry, University of Notre Dame, Notre Dame, Ind.

Infrared spectra of nitropentamminecobalt(III) chloride and nitritopentamminecobalt(III) chloride in the solid state have been measured. Spectral evidence is presented to support the conversion of nitritopentamminecobalt(III) chloride to nitropentamminecobalt-(III) chloride. In these complexes absorption bands due to NH_3 deformation vibrations—that is, degenerate, symmetric, and rocking—are identified. The remaining absorption bands in the 2 to 15 micron region can be assigned to the nitro and nitrito frequencies. Changes in the spectrum of the nitritopentamminecobalt(III) ion with regard to intensity of certain bands with time are significant. Bands which show such changes in absorption are assigned to those parts of the complex which are responsible for nitrito and nitro vibrations. By comparison of the spectra of the pure nitro- and nitrito-isomers, the bands that decrease in intensity can be assigned to the nitrito group, while those new bands that appear and gradually increase in intensity can be assigned to the nitro group.

Infrared Spectra of Coordinated Organic Ligands. BURL E. BRYANT, The University of Oklahoma, AND W. CONARD FERNELIUS, The Pennsylvania State University, State College, Pa.

The ability of the spectroscopist to detect both inter- and intramolecular hydrogen bonding in organic compounds has been of considerable aid in elucidating the behavior of a large number of compounds. For enolic compounds, the effect is most readily detected in the carbonyl region, and manifests itself by a marked shift of the carbonyl frequency toward longer wave lengths. The ability of a large number of enolic ions to coordinate with metal ions is well known. Previous (somewhat fragmentary) examinations of the infrared spectra of such metal complexes had indicated that coordination with a metal ion causes a shift in the carbonyl frequency, but no pattern had emerged from such studies.

This paper presents a study of the infrared spectra of metal complexes of two general types. In the first type studied, the organic ligands are all derivatives of tropone and in the second type, the ligands are quinones. In all cases, the ligand is of the "chelate" type, and the chelate ring contains either five or six atoms.

The effects observed are discussed in terms of the size of the chelate ring, the nature of the metal, the nature of the anion, and the nature and position of substituents on the organic ring.

Raman Spectra and Structure of Soda-Silica Glasses. R. C. LORD AND G. B. WILMOT (present address, U. S. Naval Powder Factory, Indian Head, Md.), Spectroscopy Laboratory and Department of Chemistry, Massachusetts Institute of Technology, Cambridge 39, Mass.

Raman spectra, including quantitative intensity measurements, were determined for quartz, vitreous silica and seven soda-silica glasses from 9 to 44 mole % Na_2O. Frequency and intensity distributions in the spectrum were distinctly different for quartz and vitreous silica below 500 cm.$^{-1}$, but the integrated intensities were about the same. The continuum in the spectrum of vitreous silica below 500 cm.$^{-1}$ appears to indicate that microscopic order, if any, is limited to dimensions no larger than a few times the unit-cell size of quartz.

In the spectra of the soda-silica glasses, bands attributed to the three-dimensional silica network were found to decrease in intensity with increasing soda content, while bands ascribed to different Si-O$^-$ linkages progressively increased. Frequencies characteristic of bending and stretching modes of vibration of these linkages have been assigned. The infrared spectra of the same glasses are considerably different, which seems to imply some sort of pseudo-symmetry in the silicate groups formed by the addition of soda.

Quantitative Spectrochemical Evaluation for Small Sampling Areas of Steels. H. T. GRENDON AND H. L. LOVELL, College of Mineral Industries, The Pennsylvania State University, State College, Pa.

In certain research considerations it becomes necessary to make quantitative studies of small selected areas within a steel sample. Common techniques for production analyses require pin or disk specimens of specific size and are designed to indicate an over-all composition. Diffusion studies (as for boron in steel) have been designed which demand spot analysis several times each millimeter along the length of a rod. Studies of nonhomogeneity require quantitative data of extremely small masses from micro areas of interest. Several approaches are considered, including excitation of accurately machine specimens, solution and briquetting techniques.

Methods and Standard Samples for the Spectrochemical Analysis of High-Speed Tool Steels. ROBERT E. MICHAELIS, CHARLES H. CORLISS, AND BOURDON F. SCRIBNER, National Bureau of Standards, Washington, D. C.

The spectrographic and spectrometric analyses of high-speed tool steels (W-Mo-Cr-V-Co steel) have been investigated in conjunction with the preparation and certification of six standard samples designed for this analysis. Procedures employing spark and arc excitation with both point-to-point and point-to-plane techniques are described. Spark excitation conditions may be selected to permit intercomparison of samples of different alloy types with one set of analytical curves. The effects of heat treatment as well as the conditions for highest precision in the analysis of individual types of steel are discussed. Analytical line pairs are given for the determination of Mn (0.1–0.5%), Si (0.1–0.6%), Cu (0.05–0.2%), Cr (2.0–8.0%), V (0.6–3.1%), Mo (0.7–8.5%), W (1.7–18.5%), and Co (2.5–12.0%).

Spectrochemical Analysis of Steel with the Rotating Electrode. J. P. PAGLIASSOTTI, Research Department, Standard Oil Co. (Indiana), Whiting, Ind.

A simple, rapid, and accurate rotating-electrode procedure for the spectrochemical analysis of steels in acid solutions has been developed. Condensed-spark excitation is used. Chromium, copper, manganese, molybdenum, nickel, silicon, and vanadium are determined with a precision of 2 to 4%.

The procedure is particularly suited to the needs of the steel consumer because he can not control the physical form or metallurgical history of his samples. The steel producer may find the procedure useful for classifying scrap. Extension to the analysis of samples of other metals and alloys is possible.

Analytical Studies of the Spectrochemical Determinations of Magnesium in Iron Using Solution Techniques. WM. J. EDGAR AND RUDOLPH RUST, Manufacturing Research Department, Ford Motor Co., Dearborn, Mich.

In this study a technique known as the impregnated filter paper method has been utilized which is discussed as to reproducibility and general applicability for the spectrochemical determination of magnesium in iron.

Investigations have been made as to the proper method of taking the sample into solution. The effects of certain alloying elements have been noted and evaluated.

The range covered is from about 0.0020% to 1.00% magnesium in iron. The lower limit is at present determined by the purity of the electrode material. Synthetic standards were used and the magnesium impurity in the base material was evaluated.

Spectrographic Analysis of a Nickel Base High Temperature Alloy. P. V. MOHAN AND T. P. SCHREIBER, Research Laboratories Division, General Motors Corp., Detroit, Mich.

A single exposure point-to-plane spectrographic procedure has been developed for the analysis of a nickel base high temperature alloy. Analytical curves have been established for the major constituents, Cr, Fe, Mo, Al, and Ti and the minor elements, Mn, Si, and B. High repeatability and minimum matrix effects were obtained through the use of nitrogen in the analytical gap, and through a careful choice of line pairs. Line pairs were selected for minimum interference by checking lines with an echelle spectrograph, for minimum matrix effect by careful matching of excitation potential, and for increased repeatability by keeping the individual members of a pair close together in wave length. Effects of source condition, electrode shape, and atmosphere on the preburn were investigated.

A Cooperative Study on Universal Semiquantitative Spectrochemical Methods. Study Group VI, Sub-Committee II, ASTM E-2. D. L. FRY, Chairman, Research Laboratories Division, General Motors Corp., Detroit, Mich.

This is a report of five years activity of an ASTM Study Group. The purpose of the group is to submit one or more spectrochemical methods of analyzing miscellaneous samples for all metallic constituents. The first part of the study involves a literature survey and the results of numerous personal contacts. The second part involves a cooperative study by six laboratories on six specific samples. This paper discusses the results of the cooperative tests, evaluation of the various methods, and the future plans of the Study Group.

Development of an Accurate and Rapid Method for the Direct Spectrochemical Determination of Metals in Oil. D. L. FRY, H. D. NINE, AND J. J. SCHULTZ, Research Laboratories Division, General Motors Corp., Detroit, Mich.

Continuous development since 1946 has led from a sulfate residue technique to a porous cup technique, to a rotating disk procedure, to a rotating platform method. A comparison is made of the relative value of each method in regards to minimum detectability, accuracy (ability to overcome matrix effects), repeatability, ease of procedure, and speed of analysis. ASTM, D-2 Subcommittee has set up five study groups under Section J of Research Division III to study various methods with the intention of specifying one or two as recommended procedure. In the presentation of this paper the interest of these groups is kept in mind.

Quantitative Spectrographic Determination of Vanadium in Petroleum Products Logarithmic Sector Method. J. A. KANEHANN, Socony-Vacuum Laboratories, Brooklyn 22, N. Y.

Vanadium, even though present in very small quantities, may cause serious problems in the refining of oil and in industrial boiler and gas turbine operations as it contributes to catalyst poisoning, corrosion, and combustion deposits. A rapid spectrographic method for determining vanadium in crude oils, residual fuels, and charge stocks, is described. The concentration range covered is 1.56% vanadium in the ash. Results are repeatable to about 35% of the amount present (with 95% confidence), which is comparable to the precision obtained using a previous densitometric method. The method makes use of a densitometer unnecessary and requires only 1 man-hour per sample.

A 50-gram sample is ashed and the ash is mixed with an internal standard (titanium) and buffer (silica-graphite). After arcing the sample and developing the plates, line lengths are determined with a modified optical magnifier. Actual concentrations are obtained from a calibration curve where log concentration is plotted versus line length differences (vanadium 2977.5 line–titanium 2956.1 line).

Spectrographic Analysis of Impurities in Selenium. HARRY T. DRYER AND MARY XEROCOSTAS, Westinghouse Electric Corp., East Pittsburgh, Pa.

A spectrographic method is described for the quantitative analysis of 16 impurity elements in metallic selenium. The method involves burning the sample as a fine powder in a 250-volt direct current arc. A variety of electrode shapes and photographic emulsions are necessary to determine the elements in the desired ranges. Synthetic standards were used and their preparation is briefly described. The elements determined, their analytical lines, the concentration ranges, and the precision obtained are presented.

A Simple Grating Spectrometer for the Region from 25 to 100 Microns. EARLE K. PLYLER AND NICOLO ACQUISTA, National Bureau of Standards, Washington, D. C.

A Perkin-Elmer infrared spectrometer has been converted to a grating instrument for measurements in the long wave-length region by using small gratings with 320 lines per inch and 180 lines per inch. The ruled surfaces are $2^1/_2$ by 3 inches. The stray radiation and higher orders of the gratings have been removed bythe use of roughened mirrors or small gratings with 15,000 lines per inch. Also the selective reflection from NaCl and KBr have been employed to reduce stray radiation. A thermocouple with a CsI window is used as the detector to 60 microns and another one with a crystal quartz window is used for wave lengths greater than 60 microns. High resolution is obtained in the region from 25 to 50 microns when the measurements are made in the second and third orders of a 320 lines per inch grating. A calibration of the instrument was made by using the known wave lengths of the pure rotational spectrum of water vapor. Illustrations of spectra measured with the instrument are given.

High-Resolution Spectroscopy in the 3- to 5-Micron Region with a Small Grating Spectrometer. R. C. LORD AND T. K. MCCUBBIN, JR., Spectroscopy Laboratory, Massachusetts Institute of Technology, Cambridge 39, Mass.

In continuation of work previously reported a 150-lines-per-mm. Bausch and Lomb grating has been installed in a Perkin-Elmer Model 99 double-pass monochromator. With a lead telluride cell as detector and a selenium-coated germanium filter to remove higher orders, high-resolution spectra have been measured over the range 3 to 5 microns. Rotational fine structure spacings of 0.3 cm.$^{-1}$ or less have been resolved, and wave numbers measured with a precision of 0.1 cm.$^{-1}$ or better. Calibration was done by means of higher orders of atomic lines, chiefly neon and argon. Precision of the measurements was sufficient to demonstrate that published data on methane and carbon dioxide in this region contain random errors. As an example of the usefulness of the instrumentation, it may be mentioned that rotational fine structure in cyclopropane and cyclopropane-d_6 has been easily resolved and the C—C distance in the molecule determined as 1.524 ± 0.014 A.

A New Infrared Spectrophotometer. A. C. MENZIES, Hilger and Watts, Ltd., London, England.

A description is given of a double-beam-in-time infrared spectrophotometer which can also be used for single-beam work such as, for example, the recording of infrared emission spectra.

It uses a large prism and this is available in a number of materials, but it is also arranged so that a plane grating may be used instead of the prism as the dispersing means.

All parts are robust. The absorptions which may arise due to water vapor and carbon dioxide in the air are avoided by flushing the instrument with dry air.

The change-over from double to single beam working can be made in one second.

The pre-monochromator space is not confined and contains a prefocus, with plenty of room for introducing large specimens. This renders the instrument flexible so that it can be adapted to a variety of uses.

The Use of Diffraction Gratings in Perkin-Elmer Monochromators. E. H. SIEGLER, JR., Perkin-Elmer Corp., Norwalk, Conn.

The optical considerations involved in converting a standard prism instrument to a grating instrument are reviewed and performance characteristics of such instruments are discussed.

Differential Analysis with the New Model 455 Baird Associates Spectrophotometer. DAVID Z. ROBINSON, Baird Associates, Inc., Cambridge, Mass.

Differential analysis, the method of spectrophotometry which compares two materials of similar composition, is discussed. The errors and accuracy depend on such things as pen line widths and other intangibles not often applicable to mathematical discussion.

Representative analyses and features required for good analyses are described.

Differential Analysis with Infrared Spectrophotometers. H. HAUSDORFF, H. STERNGLANZ, AND V. Z. WILLIAMS, The Perkin-Elmer Corp., Norwalk, Conn.

One of the greatest limitations in infrared spectroscopy is the determination of components present only in small amounts. The lowest limits of detectability are usually in the 0.1 to 1% concentration range by ordinary methods.

The differential technique which consists of comparing the material containing the small impurity with the major component under longer path length conditions allows for improving lower limits of detectability by a factor of 100 or more. This is generally achieved at the sacrifice of some transmitted energy, which, however, can be made up by using wider mechanical slit widths on the spectrophotometer.

Results illustrated by a few representative samples and obtained on various spectrophotometric systems are presented and discussed.

An Infrared Filter. A. C. MENZIES, Hilger & Watts, Ltd., London, England.

F-center material, consisting of potassium chloride or potassium bromide with added potassium, was suggested as a useful infrared filter material by Friedman & Glover.

This material (and other similar ones) has been made and tested in the Hilger laboratories, and Gaunt & Burns have shown that heat-treatment can influence the wave length dependence of the absorption, so that filters with controlled cut-off points can be made.

The latest results achieved with such materials will be described.

The Use of Infrared in the Smaller Industries. HENRY J. NOEBELS, Beckman Instruments Corp., Fullerton, Calif.

In the past 10 years infrared methods, particularly in the rock salt region of the spectrum, have been used extensively in routine control and research by medium and large companies. The smaller companies, and even the smaller industries to a certain extent, have not taken advantage of this technique. Originally this was, no doubt, due to the price of adequate instrumentation coupled with the large amount of investigative work that had to be accomplished before the technique could be used to advantage.

Now, however, the situation has changed. There is a great deal of information pertaining to techniques, accuracy, assignment of bands, and libraries of curves available to the user. Also, the price objection has decreased in significance since the instruments have proved their reliability, long life, and low maintenance cost.

Applications are presented demonstrating the suitability of the Beckman IR-2A infrared spectrophotometer to research, as well as control problems commonly encountered in various small industries.

Performance of a Beckman DU with Grating. WILLIAM M. WARD, Beckman Instruments, Inc., Fullerton, Calif.

A 600-lines-to-the-millimeter grating has been mounted in the DU spectrophotometer. Spectrograms are presented showing the increased resolution. New analytical applications possible with the superior resolution are discussed.

Ultraviolet Spectrophotometric Determination of Styrene and Phthalate and Fumarate Esters in Polyester Resins. R. C. HIRT, R. G. SCHMITT, AND R. W. STAFFORD, Stamford Research Laboratories, American Cyanamid Co., Stamford, Conn.

Unsaturated polyester formulations, consisting of polymeric esters of dihydric alcohols and dibasic acids dissolved in a vinyl monomer such as styrene, may be analyzed spectrophotometrically without prior chemical treatment or separation. Phthalate ester and styrene concentrations are determined spectrophotometrically, and the fumarate and/or maleate ester concentration is calculated from relations existing among the components and is used as a correction for the phthalate and styrene concentrations. The method can be of value in the rapid checking of formulations against established specifications.

The Determination of Free Phenol in Polyoxyethylene Phenyl Ethers. C. F. SMULLIN AND F. P. WETTERAU, Atlas Powder Co., Wilmington, Del.

A method is presented for the determination of free phenol in polyoxyethylene phenyl ethers. Primarily the method is based upon the ultraviolet absorption of phenol in alkaline solution. Interference by the polyoxyethylene phenyl ether is avoided by separating the phenol from the sample. The concentration of phenol remaining in an alkaline solution is obtained by measuring the transmittance at 287.5 mμ and then referring to a standard curve in which per cent transmittance is plotted against phenol concentration. The precision is on the order of 1% with an accuracy of 5% or better.

The Ultraviolet Absorbance of Thyroglobulin. CHALMERS L. GEMMILL, Department of Pharmacology, School of Medicine, University of Virginia, Charlottesville, Va.

The ultraviolet absorbance of thyroglobulin was determined using 100-mm. cells in either the Beckman DU or DK spectrophotometers. Purified thyroglobulin obtained from Kroc of Warner-Chilcott Research Laboratories was used in this work. The method of preparation and chemical analysis of this thyroglobulin has been described. A stock solution of thyroglobulin was prepared by dissolving 116 mg. of the protein in 100 ml. of $0.02N$ potassium hydroxide. When 2 ml. of this solution was diluted to 100 ml. with $0.1N$ potassium hydroxide, a characteristic ultraviolet absorbance was obtained with maxima at 291 and 247 mμ. When a similar amount of thyroglobulin was diluted to 100 ml. with $0.1N$ hydrochloric acid, pronounced differences were noted at 317 mμ and 253 mμ when this spectra was compared to the one obtained in alkaline solution. These shifts are reversible for when the alkaline solution was made acid and again made alkaline, the characteristic spectrum was obtained for the alkaline thyroglobulin. Also, curves intermediate to the alkaline and acid spectra were obtained at pH between 6.0 and 8.0. The intensity of the absorbance is proportional to the concentration of the thyroglobulin. The addition of iodine to the thyroglobulin solution increases the absorbances at 317 mμ and 253 mμ. Iodination of casein also causes an increase in these same regions. Since the region around 317 mμ is where the spectra of thyroxine and diiodotyrosine show pronounced shifts to change in pH, it is considered that the absorbances in this region may give a measure of the organically bound iodine in thyroglobulin.

Residual Styrene in GR-S Latex. A Spectrophotometric Method. C. L. HILTON, United States Rubber Co., Passaic, N. J.

A rapid method for the determination of monomeric styrene in GR-S latex is described. Styrene strongly absorbs ultraviolet energy in the wave-length region from 240 to 260 mμ depending upon the solvent used. This absorption has been utilized in the development of the present method. The accuracy and precision of the method have been found satisfactory for routine analyses. For those latices where the amount of gel present is low enough to permit direct spectrophotometric determination of the styrene in a vistex solvent such as isooctane, the sample is simply shaken with the solvent for one half hour and brought to the mark in a volumetric flask of suitable size. The absorption of the solution at three wave lengths is then utilized for the determination of residual monomer content of the latex. Lack of agreement at the three wave lengths indicates a significant amount of interference. In this case, the monomer is removed by distillation and spectrophotometric analysis of the distillate is carried out.

Advantages of the present method over the usual distillation titration method include: utilization of a smaller (0.5 rather than 25.0 grams) sample, more complete extraction of monomer from the latex, and elimination of standardization of solutions.

Gas-Liquid Partition Chromatography. D. H. LICHTENFELS, S. A. FLECK, AND F. H. BUROW, Gulf Research & Development Co., Pittsburgh, Pa.

Gas-liquid partition chromatography is a powerful technique for separating and identifying many close boiling materials. The packing material employed is a suitable granular material on which has been deposited a coating of a high boiling organic liquid such as dioctyl phthalate. When a lower boiling organic material is charged to the column it will partition between a gas phase in the pore space and a liquid phase absorbed in the organic coating. If the column is eluted with an inert gas, the material will move forward with a velocity which is less than that of the carrier gas. The velocity with which a particular component moves is dependent upon its partition coefficient. As the latter varies for different compounds there will result a separation within the column. The separated components are detected with a thermal conductivity cell as they emerge from the column.

Excellent separation between very close boiling materials corresponding to distillations of several hundred equivalent theoretical plates may be achieved. A discussion of this technique is given with examples of its usefulness as an independent analytical tool, and as a powerful adjunct to molecular spectroscopic procedures.

Methods and Results of Spectrographic Analysis of Human Blood and Tissues. N. F. SHIMP, JANE CONNOR, A. L. PRINCE, AND F. E. BEAR, Rutgers University, New Brunswick, N. J., AND H. J. KOCH, JR., AND E. R. SMITH, Sloan-Kettering Institute for Cancer Research, New York, N. Y.

Quantitative spectrographic procedures have been applied to the analysis of human tissues and blood samples. Digested samples were supplied by the Sloan-Kettering Cancer Institute and analyzed by two spectrographic procedures:

(1) The well-known Mitchell concentration procedure has been adopted to wet-digested human tissues and blood. The procedure of Mitchell has been followed in that the trace elements of the sample are precipitated from an acid solution of the sample with 8-hydroxyquinoline using Fe and Al as carriers. The variable Fe internal standard procedure of Mitchell has not been utilized, but rather small additions of Pd and Ge are made to each sample and used as internal standards. Standard curves have been prepared for Bi, Cd, Co, Ni, Zn, Pb, Sn, Ti, Cr, Ga, V, and Mo with precision that ranges near ±10%.

(2) The $NaNO_3$ buffer solution procedure as outlined by V. G. Perry, W. M. Weddell, and E. R. Wright has been adopted to wet-digested tissue and blood samples. The acid digest is taken up in 20% sodium nitrate solution and analyzed spectrographically with the alternating current arc and using Mo as an internal standard. Standard curves have been prepared for Ca, Fe, Mn, Al, Cu, K, Sr, and Mg with a precision of from ±5% to ±10%.

Results obtained in human tissues and blood by both procedures are given and certain of the spectrographic results compared with chemical analyses.

Spectrochemical Determinations of Microimpurities in Sal Ammoniac. K. W. BEYER AND O. T. AEPLI, Pennsylvania Salt Mfg. Co., Wyandotte, Mich.

A spectrochemical method was developed for the determination of low concentrations of nickel, copper, iron, and lead in sal ammoniac. The metallic impurities are fixed by the addition of 1% phosphoric acid and concentrated by the removal of the ammonium chloride by sublimation. The residue is dissolved in redistilled nitric acid and evaporated to dryness. The H_2SIO_3 is removed by hydrogen fluoride. An acid $NaNO_3$ buffer with zinc and bismuth as the internal standards is added to the residue. Excitation is obtained by a 5-ampere high voltage alternating current arc. The standard deviation for these determinations is approximately ±10%.

A Study of Matrix Effects in a Graphite Base Semiquantitative Procedure. E. L. GROVE, University of Alabama, University, Ala., AND J. A. NORRIS, Oak Ridge National Laboratory, Oak Ridge, Tenn.

This paper reports an investigation of the procedure routinely used for the analysis of electromagnetically separated isotopes and in particular the variations in analytical results due to matrix effects.

Analytical samples are mixed in a ratio of 1 to 9 parts graphite by weight and arced at 5 amperes, 2400 volts a.c. in a $^3/_{16}$-inch pedestal type electrode. Inconsistencies in analytical results for certain samples prompted the authors to study some common impurities—namely, aluminum, iron, calcium, barium, and silica in matrices involving fluorides, chlorides, carbonates, and sulfates of several alkali and alkaline earth elements which from experience have shown the greatest variations. Quantitative data showing enhancement and suppression effects are presented.

The Spectrochemical Determination of Boron in Carbon and Graphite. CYRUS FELDMAN AND JANUS Y. ELLENBURG, Oak Ridge National Laboratory, Oak Ridge, Tenn.

Moving plate studies show that the available material which best matched the volatility behavior of boron in carbon and graphite in the direct current arc was finely powdered Ir^0. When the discharge was burned in an 80-20 argon-oxygen mixture, the line-pair B 2497/Ir 2543 gave intensity ratios having a relative standard deviation of 2.5% or less between 0.5 and 4 p.p.m. boron. By use of a powder spark method, the sensitivity was extended to 0.25 p.p.m. boron. In this technique, a "sifter" electrode (a porous cup electrode with a perforated floor) is filled with powdered sample, and used as the upper electrode in a high voltage spark discharge. The opening at the top of the electrode is closed with a small cork. During the discharge, the sample material sifts into the discharge area and is excited. When this discharge was conducted in pure argon, the line pair B 2497/Cu 2492 gave a relative standard deviation of 2.5% or less between 0.25 and 3 p.p.m. boron.

Spectrographic Analysis of Glass Using the High Current A.C Arc. J. F. Bacon, H. G. Ross, and R. H. Close, Research & Development Laboratory, Corning Glass Works, Corning, N. Y.

The authors have the following objectives in presenting this paper:

To illustrate the considerable scope for the application of spectrographic methods to problems in the glass making industry.

To present a review of the techniques employed at the Corning laboratory for the qualitative, semiquantitative, and quantitative examination of glass.

To demonstrate through the presentation of typical data the advantages and limitations of the high-voltage, high-current alternating arc source for the quantitative determination of trace constituents in refractory type materials.

Quantitative Spectrographic Analysis of High Silica Minerals. S. R. Wiley, Research Center, Johns-Manville, Manville, N. J.

A direct current arc spectrographic method used in the Johns-Manville Research Center for the quantitative analysis of high silica minerals is described. A statistical comparison with accepted wet chemical methods made on diatomaceous earth samples indicates that the spectrographic method is as reliable for the determination of aluminum and iron, and is preferable for ten or more other minor and trace elements, several of which are not usually analyzed chemically because of the additional time and cost required. The silica in these samples was determined within 0.5% of true value by using the concentration-ratio principle. With a moderate reduction in accuracy, this method can be applied to the analysis of samples as small as one milligram, indicating its usefulness in the determination of the composition of small foreign silicate particles.

The Spectrographic Determination of Columbium in Silicate Ores. R. H. Dolan and A. B. Chandler, Foote Mineral Co., Philadelphia, Pa.

A spectrographic method has been developed for the determination of columbium in silicate ores. The method is direct and rapid compared to wet chemical methods. The sample is thoroughly mixed with an equal amount of powdered, pure, sugar, an internal standard, put into a pure electrode and burned in a magnetically interrupted direct current arc. The line densities are read on a densitometer, plotted and calculated for the percentage of columbium present. The method is reproducible to within ±20%.

Extended Studies on the Precision of the Jaco Spark Source with the Spark-Lock. A. J. Mitteldorf and D. O. Landon, Consolidated Testing Laboratory, Inc., West Hempstead, N. Y.

Data have been reported on the improvement of the precision of the Jaco Spark source with the Spark-Lock in the analyses of aluminum and steels. Studies have been extended to show a lower coefficient of variation on the analyses of other metals when the Spark-Lock is used.

Quantitative Absorption Studies of Oxygen and Nitrogen, 1100 to 850 A. Frederic F. Marmo, Atmospheric Physics Laboratory, Geophysics Research Directorate, Air Force Cambridge Research Center, Cambridge, Mass.

Using a vacuum monochromator, hydrogen discharge source and calibrated (absolute energy) photomultiplier tubes as detectors, the absorption coefficients of nitrogen and those of oxygen in addition to the photoionization cross sections of the latter were measured for the spectral region 1100 to 850 A. Preionization of the M, M′, H, and H′ bands of oxygen was experimentally observed. A method is described for obtaining the first ionization potentials of gaseous species. This method is spectroscopic in nature, but does not depend on the identification of a Rydberg series. Its application to oxygen yielded a value of 12.08 ± 0.01 e.v. for the first ionization potential. The combined data are applied to the theory of formation of the E layer of the earth's atmosphere.

The Ultraviolet Absorption Spectra and Basicities of Substituted Pyridine 1-Oxides. H. H. Jaffe (present address, Department of Chemistry, University of Cincinnati, Cincinnati, Ohio), Venereal Disease Experimental Laboratory, U. S. Public Health Service, School of Public Health, University of North Carolina, Chapel Hill, N. C.

The ultraviolet absorption spectra of pyridine 1-oxide and of a number of its derivatives substituted in the 3- and 4-position have been determined in water, and in mixtures of water and sulfuric acid as solvent. The spectra of pyridine 1-oxide and of its conjugate acid are compared with the spectra of pyridine and its conjugate acid. The effect of substituents on the spectra of pyridine 1-oxide and of its conjugate acid are discussed. The data are used to determine the basicities of substituted pyridine 1-oxides. The effect of substituents on the basicity of pyridine 1-oxide can be expressed in terms of the Hammett equation, using the same substituent constants used in the benzene series, and with a reaction constant $p = 2.09$, which is essentially equal to the reaction constant for the acidities of phenols.

Ultraviolet Absorption Analysis for Nitrogen Dioxide. M. S. Norris, S. A. Fleck, and D. H. Lichtenfels, Gulf Research & Development Co., Pittsburgh, Pa.

An application has been made of a procedure proposed by Frey and Moore for the ultraviolet absorption analysis of nitrogen dioxide and nitrogen tetroxide in a system containing nitrogen, nitrous oxide, nitric oxide, hydrogen, carbon dioxide and carbon monoxide.

A principal problem in this method is the experimental handling of the NO_2 and the coexistent N_2O_4. A simple procedure for calibration and testing of synthetic blends was developed using an ordinary mercury manometer, the mercury surface of which was isolated from the NO_2 by a layer of α-bromonaphthalene. Another principal problem lies in the equilibrium between NO_2 and N_2O_4 and the necessity of accurately accounting for the degree of dissociation in all stages of calibration gas and sample handling. This was done by a modification of the equations of Verhoek and Daniels and is based on their measured equilibrium constants. The NO_2 was determined by ultraviolet absorption at 394 mμ where the data of Hall and Blacet showed the other oxides of nitrogen to be transparent. The other components in the mixture are determined by mass spectrometry.

Automatic Recording of Reflectance. George P. Gale and August O. Weilbach, Beckman Division, Beckman Instruments, Inc., Fullerton, Calif.

A normal-diffuse reflectance attachment covering the visible region from 380 to 800 mμ has been developed for the Beckman Model DR quartz recording spectrophotometer enabling the automatic recording of reflectance curves. The attachment employs an integrating sphere with a specially prepared surface which does not require "smoking." It has been designed to handle a wide variety of samples—liquids, powders, solids; and it will accommodate large samples without their being cut down to fit into special sample holders. The Beckman Model DR with a special programmer and the reflectance attachment can be made to take readings only at preselected wave lengths for more routine work. The general operating characteristics and typical results are presented along with a detailed description of the attachment.

Fluorescence Spectra Excited by Ultraviolet Light and Beta Rays. Bernard Manning, Technical Operations, Inc., Arlington, Mass.

An adapter and associated instrumentation which converts the Beckman DUR monochromator to a recording fluorescence spectrophotometer is described. Transmitted and front surface spectra of a number of different organic crystals are presented and compared to the spectra obtained with beta rays. In all the examples investigated, the spectra excited by beta rays are identical with the transmitted fluorescence spectra excited by 2537 A. ultraviolet light. Absorption spectra of these crystals indicate that the missing wave bands (if any) are accounted for by self-absorption. Apparent shifts of bands are also explained by self-absorption phenomena. Spectra of solutions of these materials are also presented.

Ultraviolet Analyzer for Process Control. E. C. Miller and H. W. Staten, Jr., Research Division. Phillips Petroleum Co., Bartlesville, Okla.

In modern petrochemical processing plants automatic analyzer-controllers are increasingly necessary to maintain specification product at economic throughputs. An ultraviolet analyzer has been developed under the Office of Synthetic Rubber instrumentation research program for applications involving the analysis for butadiene in hydrocarbon streams. This device is a photoelectric photometer in which a focal isolation filter combined with a chlorine gas filter provides a suitable spectral pass band. A two-element quartz-Vycor chopper modulates the light intensity to produce an alternating current signal whose magnitude varies with the butadiene concentration. This alternating current signal is amplified by a special feedback-controlled circuit and is rectified to obtain a direct current voltage which is indicative of the butadiene concentration.

The embodiment described has a usable sensitivity of better than 0.01% butadiene at 1% butadiene and 0.1% at 10%. While this device was designed for low concentrations of butadiene, it is applicable to other concentration ranges and to other analyses.

Constant-Volume Fraction Collectors

J. Wilfrid Hahn and Melvin Nyman[1], Rheumatic Fever Research Institute, Northwestern University Medical School, Chicago, Ill.

During studies of the column chromatographic separation of urinary steroids inexpensive fraction collectors were needed to collect definite volumes of solvent. As a siphon constructed with an air escape vent (*4*) gave very good reproducibility (in a typical case, the volume of benzene determined by six weighings was 6.05 ± 0.01 ml.) it was decided to use this as a basis for the modification of a timer-actuated collector (manufactured by C. R. Stryker, Norwalk, Conn.) already in use in the laboratory and the construction of a photoelectrically controlled collector. The modification of the timer-actuated collector to deliver constant volumes is offered as a general solution of this problem for collectors that require but a single impulse to index the table. The second collector was constructed on the basis that more light is transmitted across glass tubing filled with solvent than empty tubing.

GRAVITY-ACTIVATED COLLECTOR

The addition of a latch relay and a siphon on a balance arm to the motor-driven turntable of the timer-actuated collector modified the apparatus to provide for constant volume collections (Figure 1). The principle used is essentially that described by Brimley and Snow (*1*), Chapon (*2*), and Mader and Mader (*6*). Certain modifications of a mechanical and electrical nature have been adopted to ensure ruggedness of the instrument, reasonable sensibility, a free interchange of siphons, and safe operation in the presence of flammable solvents. The use of ball bearings in place of knife-edges for pivots by kinematic principles provides for movement of the balance arm and the siphon in the desired plane of motion with very little friction and considerable ruggedness.

The siphon was suspended from one end of a balance beam equipped with two small mercury switches near the pivot and a counterbalance at the other end. An additional weight perpendicular to and above the mid-point of the beam was used to raise the center of gravity, thus placing the balance in metastable equilibrium. To maintain the siphon in vertical position the siphon holder was pivoted.

In operating the unit, the position of the counterbalance is adjusted so that the siphon arm of the balance tips downward when the siphon is half full. (In practice 1 drop of solvent is sufficient to put the balance in either extreme position, its swing being limited by mechanical stops.) In the downward position the motor circuit is closed. The motor turns two cams; one indexes the table by engaging pins projecting from it, and the other cam closes the microswitch momentarily. Closing this microswitch circuit even momentarily is sufficient to break the motor circuit, thus stopping the motor. This circuit is kept open by means of the mechanical catch within the latch relay until the siphon empties and the balance beam tips upward. This operation disengages the catch and the motor circuit is simultaneously closed within the relay and opened within the mercury switch on the balance arm to revert to the initial starting conditions. The motor indexes the table one tube only when the siphon tips downward.

A lower limit of volume of about 1.5 ml. appears to be necessary for successful operation of this equipment. This limitation may be serious where microanalytical procedures are necessary. The minimum volume seems to be determined by the mercury switches and by frictional forces in the ball bearing employed as a pivot. This disadvantage can probably be minimized by the use of corrosionproof pivot points and by replacement of the mercury switches by a photoelectric cell arrangement to indicate the posi-

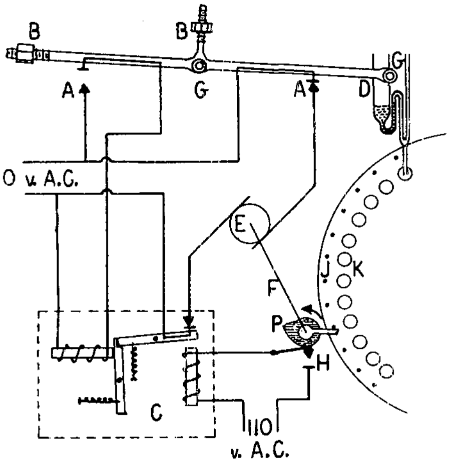

Figure 1. Schematic diagram of gravity-actuated collector

A.	Mercury switches, microswitch Type AS408A1
B.	Counterbalance weights
C.	Potter-Brumfield latch relay
D.	Siphon
E.	Motor
F.	Motor shaft
G.	Ball bearing pivots
H.	Snap action microswitch
J.	Pins projecting from table
K.	Test tube rack
P.	Cam

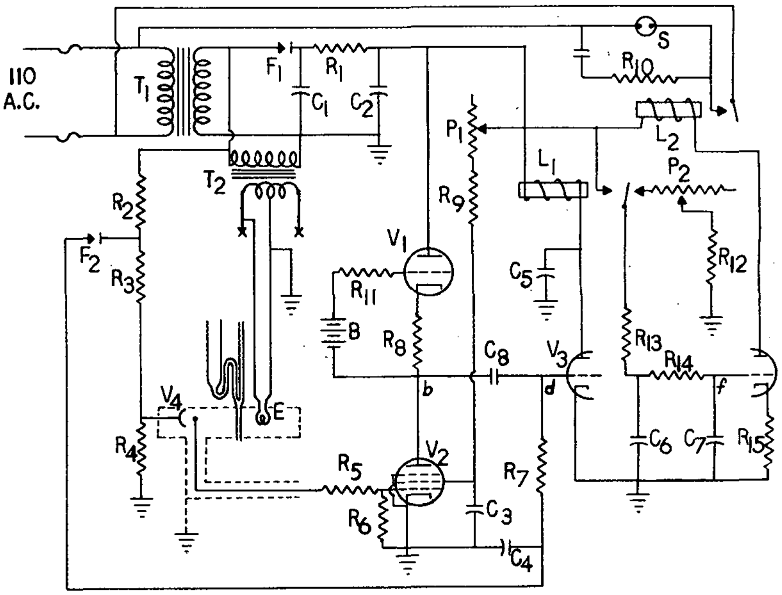

Figure 2. Electronic circuit for optically controlled collector

B.	Battery, 22.5 volts	R_2.	100,000 ohms
C_1, C_2.	15 mfd., 150 volts, elect.	R_3, R_{15}.	10,000 ohms
C_3.	0.1 mfd., 200 volts	R_4.	2,000 ohms
C_4, C_7, C_8.	1 mfd., 200 volts	R_5, R_{12}, R_{13}.	1 megohm
C_5.	10 mfd., 150 volts, elect.	R_6, R_7.	10 megohms
C_6.	2 mfd., 200 volts	R_8.	240,000 ohms
E.	Mazda flashlight bulb PR 6	R_9.	0.5 megohm
F_1.	Selenium rectifier Mallory 6S100	R_{10}.	100 ohms
		R_{11}.	150,000 ohms
F_2.	Selenium rectifier Federal 1002	R_{14}.	3 megohms
L_1, L_2.	Relay, Potter-Brumfield LM5, 10,000 ohms	T_1.	Transformer, Sola 30498
		T_2.	Transformer, UTC S 54
P_1.	Potentiometer IRC 11-139, 2 megohms	V_1.	6SL7GT
		V_2.	12SH7
P_2.	Potentiometer IRC 11-143, 10 megohms	V_3.	5692
		V_4.	1P42
R_1.	2700 ohms (all 0.5 watt)		

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[1] Present address, Southwest Foundation for Research and Education, San Antonio, Tex.

tion of the balance, a system which would not interfere with the balance properties of the beam itself.

OPTICALLY-CONTROLLED COLLECTOR

In the second collector the same type of siphon was employed. Instead of using the weight of the solvent and the movement of a balance arm to initiate the action, the siphon was fixed in a position so that the delivery arm interrupted a light beam impinging on a phototube. Because the siphon is fixed, provision can be made to minimize evaporation of the solvent by the technique of Mader and Mader (*7*) or under more serious conditions by the use of refrigeration coils. When the siphon delivers, and solvent passes through the delivery tube, more light is transmitted onto the phototube. This effect, suitably amplified, energizes a solenoid-ratchet device to turn the table (Figure 2).

Intensification of the light which falls on the phototube, V_4, makes the control grid of V_2 more negative. This causes the potential at *b* and correspondingly at *d* to become more positive, permitting a flow of current through one half of the 5692 tube, V_3, to close the LM5 relay, L_1. With the relay closed, grid *f* of the other half of the 5692 tube is now connected to the positive power supply and current flows through the tube to close relay L_2 and consequently energize the electromagnet, *S*. The electromagnet is maintained in this condition as long as liquid is passing through the delivery arm of the siphon and for an additional time (to allow complete drainage of the solvent) determined by the R-C constants of the system, C_6, C_7, R_{12}, R_{13}, R_{14}, and P_2, through which the grid is grounded. The time delay can be varied by adjustment of P_2. Final release of the electromagnet causes a spring-driven mechanism similar to that described by Schram and Bigwood (*8*) to rotate the table to its next position.

P_1 is adjusted by trial and error with the siphon in position, so that the presence or absence of solvent will determine the opening and closing of relay L_1.

The circuit described is essentially that of Johnson (*5*). It has been modified by the addition of the 6SL7GT tube, V_1, with its own separately biased grid potential as a "cascode" amplifier (*3*), to provide a constant load for the 12SH7 tube with a resultant increase in gain and stability. The system works very well in this case, where the relays must be operated by small differences in light intensity relative to the light levels used. It was not necessary to change the settings during 6 months when the collector was in nearly constant operation.

ACKNOWLEDGMENT

The expense of this investigation was covered by a grant from The Helen Hay Whitney Foundation, which is gratefully acknowledged.

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Fermentation of Sugars by an Ultramicrotechnique Prior to Paper Chromatography

Kenneth T. Williams and Arthur Bevenue, Western Utilization Research Branch, Agricultural Research Service, U. S. Department of Agriculture, Albany 6, Calif.

THE authors, continuing work on the unfermentable sugars in vegetables [Williams, K. T., Potter, E. F., and Bevenue, A., *J. Assoc. Offic. Agr. Chemists*, **35**, 484 (1952)], have developed a technique for fermenting very small volumes of sugar solutions with commercial baker's yeast. A recent article by Porter and Hoban on enzymatic hydrolysis [Porter, W. L., and Hoban, Nancy, ANAL. CHEM., **26**, 1846 (1954)] has prompted a description of the procedure as a further supplement to ultramicrotechniques for paper chromatography.

A melting point tube (1+ mm. by 100 mm.) was heated at the center and drawn into two test tubes with a bead formed on the bottom of each tube. Medicine droppers were modified by drawing the tips to a capillary to fit easily into the micro test tubes. Holes about 2 mm. in diameter were drilled into wooden or metal blocks to support the test tubes in an upright position. Small stirrers were made from drawn glass rod.

Three milliliters of acetate buffer and 3 ml. of 0.2*M* potassium dihydrogen phosphate were included in 100 ml. of a 10% washed baker's yeast suspension [Williams, K. T., and Bevenue, A., *J. Assoc. Offic. Agr. Chemists*, **36**, 970 (1953)].

The sample to be fermented, 0.01 to 0.05 ml., was introduced into the bottom of the micro test tube by means of the special eye dropper. Any solution adhering to the wall of the test tube was centrifuged, or jarred by tapping, into the liquid at the bottom of the tube. The yeast suspension, equal in volume to the sugar solution, was added with a modified eye dropper near the top of the test tube. The tube was then centrifuged, or jarred by tapping, until the yeast mixed with the sugar solution. Yeast suspension added near the top of the tube washed down any sugar solution remaining on the walls of the tube. A stirring rod was inserted in the tube and the solution was gently stirred.

The sugar and yeast mixtures were fermented in a moist atmosphere at 30° C. The solution was agitated with the stirring rod, which remained in the tube, at frequent intervals. Three per cent solutions of fructose and sucrose can be completely fermented in 4 to 6 hours. The more frequent the stirring of the yeast, the more quickly the fermentation is complete.

At the end of the fermentation period, determined by experiment with known sugar solutions, the yeast was suspended by stirring, the bead was broken from the end of the tube, and spots were made on the chromatographic paper with the tube as with a micropipet. The chromatograms were then completed in the usual manner. The yeast and the small amount of acid and salts used for buffer did not appreciably interfere with the chromatographic separation of the sugars.

Vertical Umbrella-Type Agitator to Promote Smooth Boiling in Vacuum Distillation

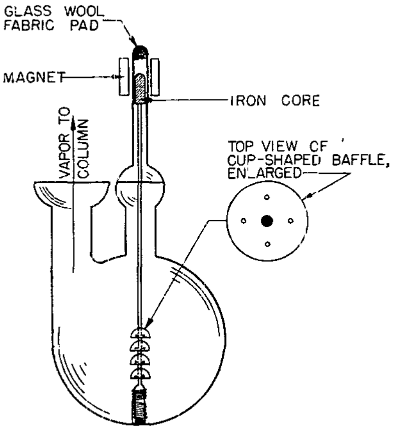
Arthur Rose and William W. Sanders, The Pennsylvania State University State College, Pa.

AN UMBRELLA-TYPE agitator has been developed to promote smooth boiling in vacuum distillation.

It consists of a vertical glass rod with glass inverted cup-shaped baffles sealed to the rod at intervals along its length. Several holes are drilled in each baffle. The upper end of the vertical shaft consists of glass tubing, within which is a close-fitting soft iron core. To the bottom end of the shaft is attached a stainless steel bumper spring, which prevents damage to the agitator and the pot when the device is operating. Minor modifications permit location of the spring at the upper end of the agitator. When the external magnet is energized, the agitator moves upward, and when the circuit is broken it falls under the influence of gravity. A variable timer permits the number of strokes per unit time to be varied. A rate of 50 to 150 strokes per minute gave smooth steady boiling for all the liquids tested. The length of the stroke was such that at least one baffle broke the liquid surface regardless of the liquid level. A baffle spacing of 1 inch was satisfactory with 5-liter still pots. Heat was supplied by a standard Glascol mantle.

All the fatty acid esters and many of the acids and alcohols with an even number of carbon atoms from C_6 to C_{18}, inclusive, have been boiled successfully at high rates of vaporization and for long periods of time, at pressures that varied from 1.0 to 735 mm. of mercury. Water was present in some instances. The device has made possible the smooth steady boiling of methyl oleate and similar mixtures at a head pressure of 1 mm. of mercury for 170 hours.

Porcelain chips, broken glass, Boileezers, wooden chips, and an external hot spot could not be used where high rates of vaporization were required for lengthy periods. Internal hot spots (*1*) were not used because of concern as to possible decomposition. The use of a capillary bubbler was avoided because of the difficulty of obtaining completely dry and oxygen-free nitrogen or other inert gas and the uncertainty as to whether the presence of inert gas changed the phenomena of distillation under study.



The most satisfactory solenoids are those made for use with steam-duty electric valves (Magnatrol Valve Corp., 68 Fifth Ave., Hawthorne, N. J., Type 18A-42 solenoid coils for Magnatrol valve for steam service, with metal case wall). A coil of copper tubing may be made just to fit around the solenoid, and cooling water can then be circulated through the copper coil. The most satisfactory timer was the Model A1 Repeat-Cycle Timer with a terminal strip (C. G. Wilson Co., Chatham, N. J.).

The effectiveness of the agitator is believed to depend on continuous formation of new surface. On the upward stroke the baffle passes through the surface of the liquid and causes a new liquid surface to be formed. The downward stroke produces the same effect, and a small quantity of vapor is trapped beneath the baffle and escapes through the holes in a stream of tiny bubbles. This again causes a new liquid surface to be formed. Hickman and coworkers (*2*) found that the rate of vaporization from a newly formed liquid surface is much greater than from an "old" surface. A rotary stirrer (*3*) also gives this effect to a limited degree.

In one test without the agitator, it was impossible to achieve smooth boiling over any appreciable period of time, with a 1-liter portion of commercial capric acid at 10 mm. of mercury pressure, regardless of the heat input. With the agitator, smooth steady boiling occurred for 14 hours, after which the test was stopped. The boil-up rate was approximately 2 or 3 liters of liquid per hour. A very marked decrease in rate was easily observed by watching the condensation ring in the condenser just after the agitator was momentarily stopped after smooth boiling had been obtained. The vapor stream practically ceased within 2 seconds after the agitator had been shut off and the liquid started to superheat. When the agitator was started again (before the first bump), rapid vaporization again commenced on the first stroke at an even faster rate than originally, until the superheat had been discharged.

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A report of work done under contract with the U. S. Department of Agriculture and authorized by the Research and Marketing Act of 1946. The contract is being supervised by the Eastern Utilization Research Branch of the Agricultural Research Service. An application has been made for a patent covering the device described.

New Technique for Two-Dimensional Descending Paper Chromatography

Mas Yamaguchi and F. D. Howard, Department of Vegetable Crops, University of California, Davis, Calif.

In two-dimensional descending paper chromatography large cabinets are desirable, and often corrosive solvents are used, requiring the use of glass or stainless steel-lined cabinets which make the setup expensive. With large chambers, it is difficult to clean and aerate the system when solvents are changed for the second dimension. The method used in this laboratory makes use of a glass jar which occupies little space, is relatively inexpensive, and can be easily set up.

Apparatus. A 12 × 24 inch plain cylindrical borosilicate glass jar is used for the chamber (Figure 1). The solvent tray is a 6-inch borosilicate glass pie plate (edge not fluted) which is supported in a 5-inch (125-mm.) funnel. The funnel on a rubber stopper is supported in a 250-ml. graduated cylinder, and the height can be adjusted to accommodate the length of the paper. The cylinder is placed on a glass plate and the 12 × 24 inch glass jar is inverted over the entire assembly. A soft rubber ring serving as a gasket between the jar and a glass plate works best to make the system airtight, but Vaseline or silicone grease may be used.

Procedure. The material to be analyzed is placed near a corner about 4 to 5 inches from the edge of an 18.25 × 22.5 inch sheet of filter paper. Cuts are made at about 1.25-inch intervals to a depth of 3 inches along the 18-inch side. These 1.25 × 3 inch tabs serve as wicks for the solvent and are folded back at the end of the cuts (Figure 2, *a*). A cylinder with a diameter slightly larger than 6 inches is made from the filter paper sheet with two stainless steel wires formed into staples. The length of the staple is determined by the circumference of the pie plate, which is slightly more than 18.25 inches. The top staple is placed about 5 inches down from the top edge of the paper (2 inches from the end of the wick) and the other staple about 1 inch from the bottom. The filter paper cylinder is slipped over the pie plate, and the wicks are arranged so that they are positioned on the bottom of the pie plate. An inverted stemless 100-mm. funnel holds the wicks in place. The wicks may also be weighted down with a Petri dish, circular glass plate, or the circular base of a 500-ml. graduated cylinder cut off to contain about 50 to 75 ml. The solvent trough should be level.

The solvent is introduced into the pie plate, and the glass jar is inverted over the entire assembly. If a saturated atmosphere is desired before the solvent is introduced, beakers of the solvent

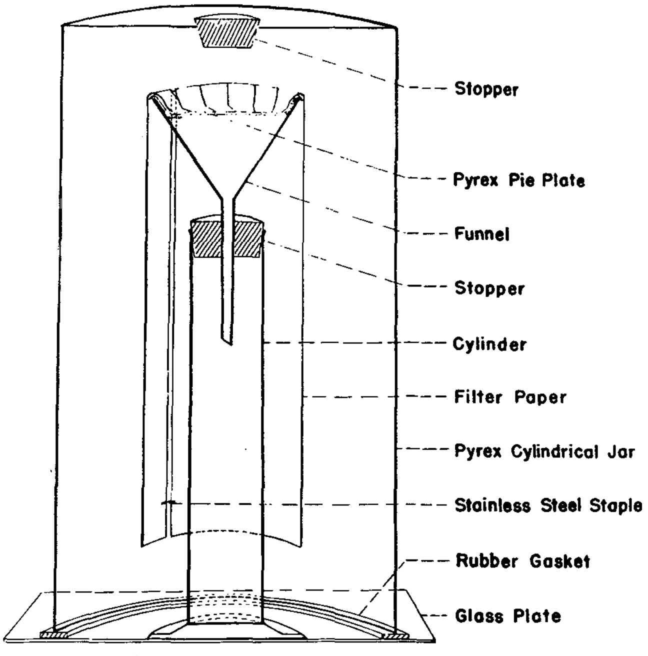

Figure 1. Cross-sectional view of setup for two-dimensional descending paper chromatography

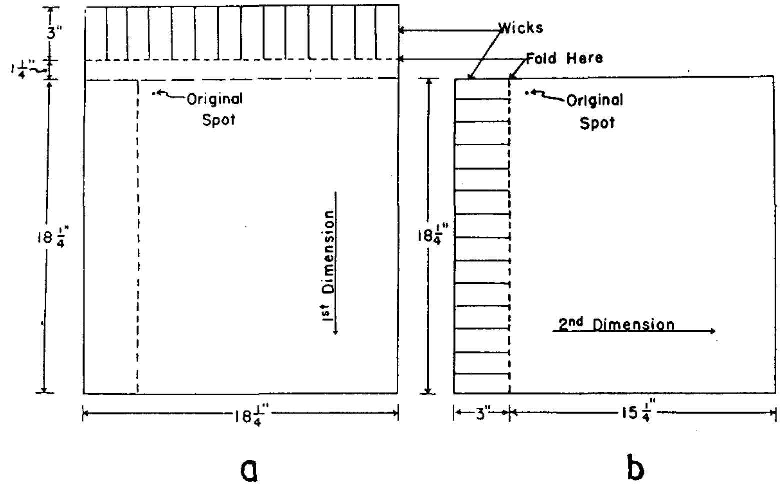

Figure 2. Preparation of filter paper sheet for first and second dimensions

may be left on the glass plate, or if the base of a graduated cylinder is used to hold the wicks in place, some solvent may be put in the cylinder. In this case, after saturation has been reached, the solvent is introduced into the pie plate through a hole drilled in the bottom of the jar. The trough should be about half full. At first the solvent front appears somewhat scalloped, but after about one inch of descent, the front straightens out. This scalloped front may be minimized by wetting the wicks uniformly when the solvent is introduced.

After solvent development and removal of the glass jar, the weight holding the wicks in place is removed and the solvent pipetted out of the trough. The residual solvent is removed by absorption with several sheets of circular filter paper and discarded. The paper is then dried in position by moving the assembly on the glass plate to a suitable drying location.

When dry, the paper can be easily removed from the assembly and prepared for the second dimension. The wicks for the first solvent are cut off, reducing the length of the paper to 18.25 inches (Figure 2, *b*). It is desirable to cut off a 1- to 1.25-inch strip of paper from the leading edge (solvent front) because some material from the paper carried down by the first solvent might interfere with the solvent descent of the second front. If this is done, the paper should be trimmed from the wick side to make the total length 18.25 inches. If the paper is more than the circumference of a pie plate, 18.75 inches, the edges may overlap when the cylinder is made. Sometimes this causes the solvent to siphon down the seam, ruining the determination.

For the second dimension, the procedure is repeated. No difference can be detected between the two-dimensional descending paper chromatogram developed by the above method and those developed in large chromatographic cabinets. This technique may also be used for one-dimensional descending chromatography.

Self-Adjusting and Dispensing Micropipet

Benjamin W. Grunbaum and Paul L. Kirk, Department of Biochemistry, University of California, Berkeley, Calif.

In this and many other microchemical laboratories, the use of capillary micropipets is extensive. Pipets of this type are usually calibrated for content, and for highest precision require rinsing before a second aliquot may be measured. In routine analysis, rinsing and drying of many pipets are time-consuming.

It is sometimes necessary to measure constant aliquots of organic solvents, as in the determination of fatty acids by potentiometric titration (*2*), and in analyses that involve very dilute reagents. Organic solvents may drain erratically, and rinsing from the filling tip is not always effective. These and other problems are readily solved by use of the special pipet illustrated in Figure 1. It is designed to be used as a dispensing pipet for successive samples drawn from the reservoir bulb, or as a measuring pipet in which constant volumes of rinse liquid can be employed reproducibly by drawing them from the reservoir bulb.

The pipet, depending on its capacity (*3*), is essentially a self-filling or a self-adjusting measuring tube with fine enough capillaries on both ends to permit adjusting the volume after filling from either end. On the top is sealed a reservoir bulb and tapered opening which fits into a syringe pipet control (Microchemical Specialties Co. type), or a rubber tube for control by the mouth. For convenience, the capillary carries a calibration mark at its center, so that the capacities above and below the mark are equal. Additional marks may be added to allow the pipet to serve for a variety of measurements, but accuracy is lost if too short a capillary is employed.

OPERATION

When the pipet is to be used for measuring organic solvents, viscous solutions, or similar materials that are difficult to remove by conventional rinsing, the operation is as follows:

The rinse liquid is placed in the reservoir bulb by means of a length of fine plastic tubing inserted through the upper opening. The sample to be measured is allowed to flow into the horizontal tube by capillary action, or is drawn in by means of a pipet control or mouth suction. It may be adjusted to the mark or allowed to adjust itself when the entire capacity of the measuring tube is utilized. During filling, the pipet is rotated so that the curved end inside the bulb opens upward out of the liquid. The sample is expelled, the pipet rotated to lower its curved tip into the rinse liquid, and the liquid allowed to fill the pipet. It is expelled after the upper tip has again been freed of the liquid. Thus, a measured amount of rinse liquid is introduced at the top of the pipet tube, carrying down with it all of the residual liquid in the pipet. The rinse liquid may be adjusted to half quantity by allowing it to flow only to the middle mark, or other variations are possible. If the pipet has been coated with Desicote (*1*), and if the liquids used are not incompatible with this material, the pipet will be dry at the end of the rinsing and can be immediately used for the next measurement. It is also possible to centrifuge the tube when viscous or organic solutions are used, as a means of obtaining complete drainage (*1*).

When used for dispensing, the liquid is placed in the upper pipet reservoir. Desicote treatment allows total drainage of aqueous solutions. The upper tip is rotated under the surface of the solution, a little pressure is applied if necessary to the upper tapered tube, and the meniscus is allowed to adjust itself to the tip. Rotation to free the tip allows delivery by a slight increase in pressure inside the bulb. If the delivery is slow and even, it will be complete, and the pipet is ready to dispense the next sample. If less than the total capacity is to be delivered, the pipet is filled only to the middle, or another, calibration mark. Centrifugation may be used with solutions that do not drain well from surfaces coated with Desicote.

Figure 1

Used either for serial dispensing of solutions, or as a convenient quantitative sampling pipet with controlled rinsing, this design allows much more rapid and simple manipulation than other designs of pipets that have been commonly used for these purposes (*3*).

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INVESTIGATION supported by grants from the National Institutes of Health, U. S. Public Health Service, and the Research Committee of the University of California.

Modified Nitrometer

B. D. Holt, Chemistry Division, Argonne National Laboratory, Lemont, Ill.

The nitrometer, accepted as standard apparatus (*1*, *3*) for the determination of nitrates, or nitrites, in mixed acids, nitrocellulose, and other organic or inorganic compounds, is commonly known as the Du Pont nitrometer. In spite of its general use, improvement has been needed to decrease its unwieldiness in operation. Elving and McElroy (*2*) described the construction and operation of a semimicronitrometer which could be either hand-operated or motor-driven. This development of a scaled-down model offered more compactness, less mercury weight, and the possibility of operating more than one apparatus at a time. The nitrometers on the market today, however, remain relatively unchanged from those of 50 years ago (*4*).

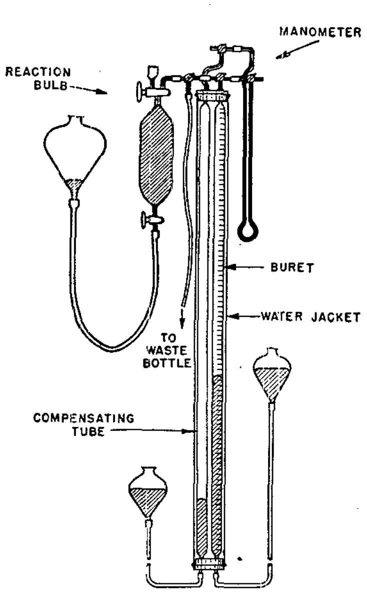

Figure 1. Modified nitrometer

At this laboratory the awkwardness of measuring the nitric oxide gas evolved in the reaction bulb has been substantially decreased by replacing the conventional gas-measuring assembly by one fabricated from a Fisher Precision Universal Model gas-measuring unit shown in Figure 1.

In this modified nitrometer the buret and the compensating tube are enclosed together in a water jacket to maintain common tube temperatures and are connected only by a mercury manometer, each tube having its own leveling bulb. After standardization, the only component of the new assembly which need be moved during a gas measurement is the leveling bulb of the buret. The correct balance with the compensating tube is indicated by the manometer. This simple manipulation stands in contrast to the multiple adjustments which are usually necessary on the conventional nitrometer. Two of the components connected to the three-branch manifold are alternately adjusted in order to balance the mercury columns in the measuring and compensating tubes, and at the same time to establish the column in the compensating tube on the standardization mark. Adjustments on these long glass tubes are clumsy because of the weight of mercury filling their lower portions and the rubber tubing connected to them.

A secondary advantage of the revised model is found in the use of the 100.0-ml. buret instead of the 240.36-ml. measuring tube. It permits the use of a smaller reaction bulb with a correspondingly smaller mercury load to be handled during the reaction. At this laboratory the reaction bulb was made from a 200-ml. gas sampling bulb by replacing one of its two-way stopcocks by a three-way stopcock and a cup as shown in Figure 1.

Although the physical connections between the measuring tube (or buret) and the compensating tube are entirely different, the principle involved in the gas measurement is the same. A certain amount of gas in the compensating tube is compressed to the volume it would have at standard conditions of temperature and pressure. The pressure of the gas in the buret is then made equal to the pressure in the compensating tube, as indicated by the manometer, thereby compressing it to the volume it would have at standard conditions. This may be shown by comparing the gas law relationships in the two tubes when the temperatures and pressures are equal. In the compensating tube when the gas is compressed to standard volume, V_C, by pressure, P_C,

$$P_C V_C = n_C R T \tag{1}$$

Similarly, in the buret,

$$P_B V_B = n_B R T \tag{2}$$

Dividing Equation 2 by 1,

$$V_B = (n_B/n_C)V_C \tag{3}$$

Since

$$V_C = 22{,}400 n_C \tag{4}$$

then

$$V_B = 22{,}400 n_B \tag{5}$$

The nitrogen constituent desired may then be calculated as follows:

$$\%\ \text{nitrogen constituent} = \frac{V_B \times \text{molecular weight} \times 100}{22{,}400 \times \text{weight of sample}}$$

STANDARDIZATION

The standardization of the apparatus is begun by drawing into the buret the nitric oxide liberated from a known nitrate sample. If, say, 0.3611 gram of potassium nitrate is used, the gas is compressed to its standard volume reading of 80.0 ml. Some arbitrary amount of dried air is then drawn into the compensating tube, so that the mercury column is only 2 or 3 inches high. Both the compensating tube and the buret are opened to the manometer. The leveling bulb of the compensating tube is moved up until the manometer is balanced. A strip of black electrical tape is wrapped part way around the water jacket, so that two points of the lower edge may serve to define the position of the upper meniscus of the mercury column. When the apparatus is in use, the mercury in the compensating tube is maintained at this position.

ANALYSIS

The sample is introduced into the reaction bulb and made to react with sulfuric acid and mercury in the same manner as with the conventional apparatus (*1*, *2*, *4*). When the reaction is complete, the evolved nitric oxide is transferred to the buret; the manometer is balanced against the compensating tube by adjusting the leveling bulb of the buret. The volume of the gas at standard conditions is read from the buret.

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