ANALYTICAL CHEMISTRY

WALTER J. MURPHY, Editorial Director

Teaching of Instrumental Analysis

At the Minneapolis (fall) meeting of the American Chemical Society, I. M. Kolthoff of the University of Minnesota presented a brief paper on the subject "Instrumental Methods in Analytical Curricula," so rich with ideas that we want to present at least some of them to our readers.

In his opening paragraph he refutes the idea that instrumental analysis is something relatively new.

"Instrumental methods of analysis is a new name for a very old subject. In quantitative analysis we make use of properties, be they chemical, physical, or biochemical, which can be measured quantitatively. The measurements are made with an instrument and therefore it is no overstatement to claim that instrumental methods of analysis is a subject as old as quantitative analysis. Instrumental methods like those involving a spectrograph, galvanometer, resistance, conductance cell, gas buret, electroscope, and spectrophotometer, have been practiced for many years . . ."

Dr. Kolthoff recognizes, as all of us do, the reasons for the phenomenal progress and advance of instrumentation. "Instruments are now being made available or are available which allow the determination of a property in considerably less time and very frequently with a greater accuracy than has been possible with the older types of instrument . . ."

One of the great dangers that Dr. Kolthoff fears is an attempt to use the college and university for the training of technicians.

"Even if one of the main aims of modern instrumentation, that of automation, were fulfilled, we still would continue to teach the theoretical and experimental fundamentals involved in the measurement of a property. It is not a function of a university to educate skilled technicians. The student should be provided with a thorough understanding of the theoretical fundamentals and be made familiar with the principles involved in the actual measurements, but the emphasis should be on the former. A course in *experimental* techniques alone without theoretical fundamentals does not belong in a university curriculum . . .

"Quite generally our aims should be to provide a thorough education in the theoretical fundamentals of methods of analysis with emphasis on the possibilities and limitations of the methods . . . From frequent requests for recommendation of Ph.D.'s who are specialists in spectroscopy, polarography, etc., it is evident that some industries are still laboring under the misconception that we provide education for specialists in certain techniques. Our educational philosophy is opposed to such training, because we teach analytical chemistry as a science and not only as a technique . . ."

These and other thoughts expressed by Dr. Kolthoff in his paper are ones that leaders in both academic and industrial circles would endorse wholeheartedly. It might be said, then, What is the point of emphasizing them?

We think it very important periodically to review basic objectives. There is a strong tendency in this day and age to stress *applied* rather than the *basic* fundamental concepts. It is relatively easy for a college or university to let itself be used by industry to train technicians. It is relatively easy to drift into this pattern unless there is a periodic review of what factors are important in the training of professional analysts.

It is our contention that the training of the technician essentially is the responsibility of industry or, perhaps, two-year schools for technicians. Definitely this responsibility should not be taken on by colleges and universities and most certainly not at the graduate level.

More and more we are witnessing in the field of analysis a sharp line of demarcation between the professional and subprofessional. This trend will grow as the field of analytical chemistry becomes more and more dependent on a wide variety of scientific disciplines. The professional reputation of the analytical chemist is improving very rapidly. Much of this change is due to the fact that management at long last is coming to recognize the distinction between the capabilities of the professional and the subprofessional in the analytical laboratory.

Application of High Frequency Methods to Detection of Bands in Partition Chromatography

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The high frequency method may be used to monitor partition chromatographic columns if conductance changes occur during elution. The advantage of the high frequency method over the low frequency method is that external electrodes may be employed. In this study a 4-Mc. tuned-grid tuned-plate oscillator was used to follow the separation of carboxylic acids. Changes in grid voltage occurring during the elution of the acids were measured. An automatically recorded chromatogram is shown. The shape of the peaks is roughly correlated with the nonlinear response of the instrument and with such factors as the total concentration of the acids in the band, distribution of the acids between the aqueous and chloroform phases, dissociation constants of the acids, and the band width.

IN MANY chromatographic separations, a change in conductance and/or capacitance is produced as a band moves down the column. This has led to the application of high frequency methods for the detection of these bands. The first application was probably made by Troitskiĭ (*10*), who used a dielectric constant sensitive instrument to detect adsorption boundaries in chromatography. Monaghan, Moseley, Burkhalter, and Nance (*8*) and Laskowski and Putscher (*6*) applied the high frequency method to adsorption chromatography by following capacitance changes in nonaqueous systems. Honda (*4*) and Honda and Tadano (*5*) used the method to monitor ion exchange columns. Hashimoto and Mori (*3*) extended the method to paper chromatography, detecting adsorption zones by passing the paper between two closely spaced metal condenser plates.

At the present time no direct application of the high frequency method of monitoring chromatographic columns has been made to partition chromatography. The high frequency method should be applicable to partition chromatographic analysis since pronounced changes in conductance sometimes occur during elution. The purpose of this paper is to describe the results obtained using the high frequency method to detect bands and to relate the response of the instrument to changes in the partition chromatographic system.

Partition chromatography is a differential migration method of analysis which is applicable to the separation of substances that can be distributed between two immiscible liquids. Successful application of the high frequency detectors to this method of analysis requires that rather pronounced changes in conductance and/or capacitance occur in the zones in which the constituents are distributed. The well known separation of carboxylic acids by the method of Marvel and Rands (*7*) fulfills this requirement.

The chromatographic separation of water-soluble carboxylic acids involves many successive distributions of the acids between two phases: the immobile phase and the mobile phase or eluent. The immobile phase consists of water which has been adsorbed on the column support; in this case silicic acid. The mobile phase is chloroform, which is made increasingly more polar by the addition of *n*-butyl alcohol at regular intervals. Acids in chloroform solution are adsorbed at the top of the column, and are then eluted with the mobile phase. The distribution of the acids between the two phases determines the rate of elution. Acids which are more soluble in the aqueous phase are eluted slowly, whereas those more soluble in the chloroform phase are eluted more rapidly.

The acids dissociate in the aqueous phase, rendering it conducting, whereas the acids are not appreciably ionized in the slightly polar chloroform phase, which is poorly conducting. Also, the total concentration of acids is so low in the chloroform phase that they exert practically no effect on the dielectric constant. It is therefore the conductance of the immobile aqueous phase which allows detection of partition chromatographic bands by the high frequency method.

Although low frequency conductometric methods might be applicable for monitoring partition chromatographic systems, the high frequency method offers definite advantages. The high frequency method permits use of external electrodes, which is a decided advantage over the internal electrodes required by low frequency methods. Making the external electrodes movable might allow the column to be scanned. The response of high frequency methods is more rapid than the response of low frequency methods, since the problem of equilibrating the solution and electrodes does not exist.

METHOD OF APPLICATION

The high frequency instrument used in this study is essentially the high frequency titration apparatus described by Hall (*1*). This instrument is a crystal oscillator with a vacuum tube voltmeter in the grid circuit, and gives an indication of both conductance changes and capacitance changes taking place between the electrodes. The instrument was found very suitable for this study. It was extremely stable over the long periods of time necessary to carry out the chromatographic analysis and had ample sensitivity.

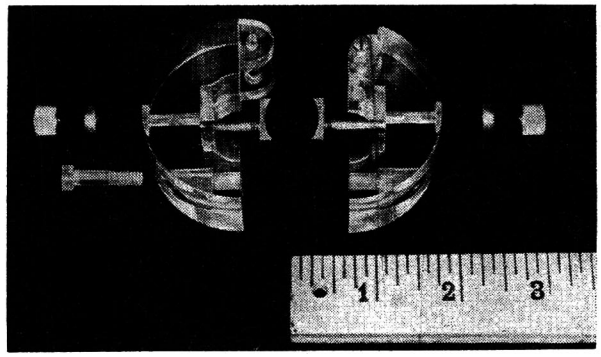

Figure 1. Exploded view of electrodes and holder

Minor changes were made in Hall's instrument. The series capacitors were eliminated, because the conductances of the chromatographic systems were within the normal range of the instrument. One of the vacuum tube voltmeter output leads was changed to the ground potential of the oscillator, so that the amplifier and oscillator could be operated from the same power supply when recording chromatograms. The oscillator was operated at 4.277 Mc., but like Hall's instrument, other frequencies could be substituted.

A 250-volt regulated power supply was used for the source of plate power, and a 6.3-volt direct current source for the filaments. The potentiometer voltage was obtained from three 7.5-volt B

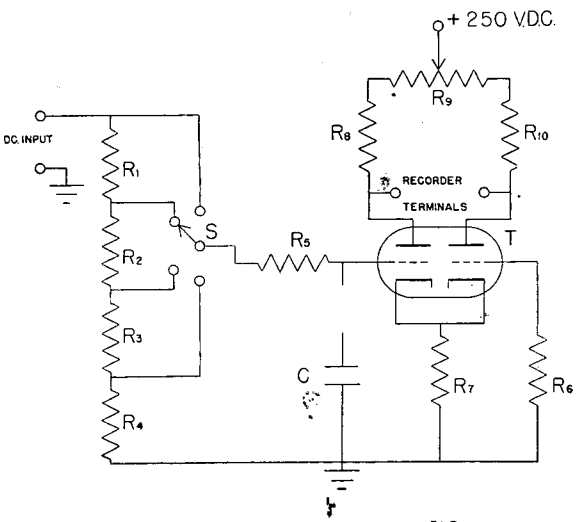

Figure 2. Direct current amplifier

C.	0.003-μfd. mica, 500 volts
R_1.	9 megohms
R_2.	910,000 ohms
R_3.	91,000 ohms
R_4.	10,000 ohms
R_5, R_6.	2.2 megohms
R_7.	600 ohms, 5 watts
R_8, R_{10}.	5000 ohms, 1 watt
R_9.	10,000-ohm potentiometer
S.	Rotary switch, 1 pole, 4 positions
T.	6SN7 vacuum tube

batteries. An RCA Model WV-97A Volt-Ohmyst vacuum tube voltmeter was used for the voltage measurements.

Electrodes for this study were specially designed to fit the column. An exploded view of the electrodes and electrode holder is shown in Figure 1. The electrode holder was made from Lucite, whereas the electrodes and the remainder of the fittings were made from brass. The only critical dimensions in the unit were the spacing and radius of curvature of the electrodes. These were machined to ensure a snug fit on the column. The hinge and clamp arrangement of the electrode holder allowed the electrodes to be clamped on the column at any desired position.

Preliminary investigations were carried out using a visual method to locate the bands on the column. This was done by adding one drop of a 0.1% methyl orange indicator solution to each milliliter of the aqueous phase and by making the aqueous phase 0.0016N in sodium carbonate. The bands could be seen easily, but no significant alteration of the chromatographic process could be detected. This technique was valuable at the beginning of the work, as it definitely associated the response of the oscillator with the passage of bands and permitted an estimation of the size and shape of the bands. In general, the bands had a sharp leading edge, and a more diffuse tail. Easily eluted acids, such as benzoic and salicylic, gave the narrowest bands. The width of these bands varied from 1.5 to 2.0 cm. under the conditions to be described.

The column used conformed to the size usually employed in laboratory separations, being 20 mm. in outer diameter and 48 cm. long. Since the bands on such a column were 1.5 to 2.0 cm. wide, the electrodes were made 1.5 cm. wide. The electrode area was about 2 sq. cm. Electrodes this small reduce the sensitivity in accordance with the theory of high frequency response (*9*).

Electrodes of the same size were also placed directly on the outer surface of the column in the form of a metallic coating. When movable electrodes were not required this method was preferred, because elimination of the air gap between the electrodes and the column gave higher sensitivity. Also, accurately machined electrodes were not required, although a holder similar to the one described above was still necessary to produce electrical contact. The metallic coating was applied by painting Du Pont Silver Paint 4666 on opposite sides of the column corresponding to electrodes. The paint was heated at 600° C. for about 3 hours to remove the binder and leave the column with a silver coating.

The chromatograms obtained in this study were automatically recorded. An Esterline Angus direct current recording milliammeter with a 0- to 1-ma. scale was used. This recorder could not be connected directly across the grid resistor of the oscillator as was the vacuum tube voltmeter, because the impedances were not matched. It was necessary to interpose the balanced direct current negative feedback amplifier, shown in Figure 2, between the grid resistor and recorder. An explanation of the operation of this type of amplifier is given in standard electronics texts (*4*).

The balanced amplifier circuit provided stability against changes in the heater voltage, cathode emission, and direct current supply voltage. A 1.2-volt input to the amplifier gave a full scale deflection of the milliammeter placed at the output. The amplifier was linear in this range. Higher input ranges were available by means of the voltage divider formed by R_1 to R_4.

The experimental procedure of Marvel and Rands was followed in every respect, except that slightly larger volumes (15 ml.) of water were adsorbed on the silicic acid. The reagents, including the silicic acid, were identical to those described by Marvel and Rands.

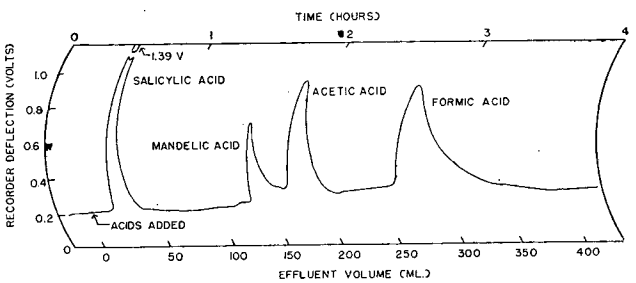

Figure 3. Recorded partition chromatogram of an acid mixture

Adsorption. 0.1 meq. each of salicylic, acetic, and formic acids, and 0.066 meq. of mandelic acid adsorbed from 1 ml. of chloroform solution

Elution

0–100 ml.	100% chloroform
100–200 ml.	95% chloroform – 5 vol. % *n*-butyl alcohol
200–300 ml.	90% chloroform – 10 vol. % *n*-butyl alcohol
300–400 ml.	85% chloroform – 15 vol. % *n*-butyl alcohol

Figure 3 is a chromatogram recorded using the described apparatus. The permanent electrodes were used. Use of the movable electrodes without the silver coating lowered the peaks by about 40%. Conditions for the separation are stated on Figure 3.

The ordinate is proportional to changes in the grid voltage of the oscillator. In recording the chromatograms, the oscillator was not tuned by the series capacitors to obtain maximum resonant voltages, and hence the changes in the grid voltage are not entirely due to conductance changes. However, it was experimentally determined that the major portion of the grid voltage change is due to conductance changes and not to changes in capacitance.

The abscissa in Figure 3 is linear with time. The effluent volume has been added to the time scale. The volume scale is approximately linear, since the flow rate was regulated at 2 ml. per minute by means of air pressure. The peaks in Figure 3 do not correspond to the peak effluent volumes described by Marvel and Rands, because the electrodes are 3 cm. from the end of the column. In general, the acids were eluted at the same peak effluent volumes as given by Marvel and Rands, except for mandelic acid, which was slightly retarded for unknown reasons.

The peak shapes may be roughly correlated with the factors affecting the response of high frequency instruments. The response depends on the average concentration of the ions in the aqueous phase between the electrodes. For a particular band, the ion concentration in turn depends upon the total amount of acid in the band, distribution of the acid between the

aqueous and chloroform phases, dissociation constant of the acid, and the band width. The effect of the dissociation constant on the high frequency response is borne out by the behavior of benzoic acid (not shown). Benzoic acid, like salicylic acid, is present in a narrow band and is eluted at approximately the same peak effluent volume indicating about the same distribution constant as salicylic acid. Unlike salicylic acid, however, the benzoic acid peak is low because of its small dissociation constant. The broadening of the bands with decrease in the rate of elution is evident from Figure 3.

It should be emphasized that the area under a peak cannot be taken as a precise measure of the amount of an acid, as the ordinate depends nonlinearly on concentration. The high frequency method is recommended only for monitoring and not for precise quantitative estimation. Also, because of this nonlinearity, the chromatographic peaks do not have the same shape as those obtained by chemical analysis of the effluent. Since the sensitivity of the high frequency method is greatest at low concentrations, the tails of the peaks are exaggerated in the high frequency chromatograms.

Changes in the base line of Figure 3 do not interfere with location of the peaks, because the change in grid voltage upon passage of a band is sufficiently large to locate the peaks. Changes in the base line are believed to be due primarily to drifting of the oscillator and changes occurring in the aqueous phase upon elution.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Benedict, R. R., "Introduction to Industrial Electronics," pp. 82, 322, Prentice-Hall, New York, 1951.
(2) Hall, J. L., ANAL. CHEM. 24, 1244 (1952).
(3) Hashimoto, Y., and Mori, I., *J. Pharm. Soc. Japan* 72, 1532 (1952).
(4) Honda, M., *J. Chem. Soc. Japan, Pure Chem. Sect.* 73, 529 (1952).
(5) Honda, M., and Tadano, H., *Japan Analyst* 2, 456 (1953).
(6) Laskowski, D. E., and Putscher, R. E., ANAL. CHEM. 24, 965 (1952).
(7) Marvel, C. J., and Rands, R. D., *J. Am. Chem. Soc.* 72, 2642 (1950).
(8) Monaghan, P. H., Moseley, P. B., Burkhalter, T. S., and Nance, O. A., ANAL. CHEM. 24, 193–5 (1952).
(9) Reilley, C. N., and McCurdy, W. H., Jr., *Ibid.*, 25, 86 (1953).
(10) Troitskiĭ, G. V., *Biokhimiya* 5, 375 (1940).

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Chromatography of Pyrimidine Reduction Products

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An analytical procedure has been developed for studying biological conversion of pyrimidines to β-amino acids through the intermediate dihydropyrimidine and β-ureido acid stages. The first two classes of compounds were detected on filter paper chromatograms by the usual methods involving ultraviolet light and ninhydrin, respectively; dihydropyrimidine rings were then opened by spraying the paper with dilute alkali, and the β-ureido acids so formed, or initially present, were detected by a final spray containing *p*-dimethylaminobenzaldehyde and hydrochloric acid. The latter reaction showed a high and rather variable sensitivity, but when one-dimensional chromatography provided adequate resolution it was relatively easy to make moderately accurate estimations by simultaneous preparation of replicate chromatograms of unknown and standard solutions.

THE initial evidence for a reductive pathway in pyrimidine catabolism (*6, 7, 11*) made it desirable to develop a sensitive and convenient procedure for separating, detecting, and estimating pyrimidine reduction products in biological mixtures. The acid hydrolysis of dihydrouracil and dihydrothymine to ninhydrin-reactive β-alanine and β-aminoisobutyric acid, respectively, provided an indirect approach to dihydropyrimidine estimation (*10*), but a much more practical and useful technique was made possible by the discovery that a spray of dilute alkali could convert dihydrouracil and dihydrothymine on intact filter paper chromatograms to β-ureidopropionic acid and β-ureidoisobutyric acid, respectively, which give a yellow color with acidified *p*-dimethylaminobenzaldehyde, as do many other compounds having the general formula $RNHCONH_2$. A procedure based on these findings has been outlined in preliminary communications (*8, 9*), and the present paper describes in greater detail investigations regarding the qualitative and quantitative capabilities of the technique.

The marked sensitivity to alkaline hydrolysis shown by dihydropyrimidines was noted independently by Batt, Martin, Ploeser, and Murray (*1*), using the decrease in absorption at 230 mμ as a measure of the extent of hydrolysis. The *p*-dimethylaminobenzaldehyde solution employed in the present studies was only a slight modification of the "Ehrlich's reagent" initially used to produce a red color with indole compounds (*5*) and subsequently adapted for a variety of other color tests, including the quantitative determination of urea in aqueous solutions (*12*) and the detection of citrulline (*4*), urea, and allantoin (*2*) as yellow spots on paper chromatograms.

METHODS

Chromatography. The equipment, solvents, and general techniques employed in preparing two-dimensional chromatograms and treating with ninhydrin were the same as those routinely used for amino acid analyses in this laboratory (*6*). One-dimensional chromatograms were prepared with the same equipment or by the ascending technique in glass jars. Solvent mixtures investigated in detail are listed in Table I.

The following procedure was ordinarily employed for color development with *p*-dimethylaminobenzaldehyde (after ninhydrin treatment, if desired). When solvents had evaporated from the paper, the paper was sprayed uniformly with 0.5*N* sodium hydroxide and allowed to dry for at least 30 minutes. The paper was then sprayed with a solution containing 1 gram of *p*-dimethylaminobenzaldehyde (recrystallized from ethyl alcohol), 10 ml. of concentrated hydrochloric acid, and 100 ml. of ethyl alcohol, dried for a few minutes in the hood, hung in a ventilated room for 2 to 6 hours (depending upon the apparent rate of color development in spots and in the reagent background), and viewed in

transmitted light to facilitate the outlining of colored spots. The sodium hydroxide spray was generally omitted if only R_f values of compounds not requiring preliminary hydrolysis were to be determined.

Table I. R_f Values of Some Substances Giving Yellow Color with Acidic *p*-Dimethylaminobenzaldehyde

Test Substance	Phenol[a]	*sec-*, *tert*-BuOH[b]	Bu-Ac[c]
Ureidosuccinic acid	0.26	0.14	0.52
Ureidoglutaric acid	0.30	0.20	0.62
Dihydroorotic acid[d, e]	0.34	0.28	0.43
α-Ureidopropionic acid	0.53	0.22	0.70
Allantoin	0.53	0.38	0.34
Isobarbituric acid[f]	0.56	0.53	0.43
Uramil	0.57	0.25	0.58
5-Ureidouracil	0.59	0.37	0.34
Citrulline	0.62	0.28	0.38
β-Ureidopropionic acid	0.62	0.22	0.65
Urea	0.75	0.49	0.57
β-Ureidoisobutyric acid	0.78	0.30	0.76
5-Aminouracil	0.78	0.45	0.36
Dihydrouracil[d]	0.90	0.55	0.56
Dihydrothymine[d]	0.94	0.71	0.69
6-Methylhydrouracil[d]	0.94	0.68	0.67

[a] Lower phase from phenol-water mixture.
[b] Upper phase from mixture of *sec-* and *tert*-butyl alcohols with water in volume ratios 5 to 1 to 5.6.
[c] *n*-Butyl alcohol, acetic acid, and water in volume ratios of 2 to 1 to 1.
[d] Preliminary spraying with alkali required.
[e] Kindly furnished by C. S. Miller, Sharp and Dohme.
[f] Barbituric acid yields an orange spot showing R_f values of 0.34 in phenol, 0.21 in *sec-*, *tert*-BuOH, and 0.44 (with streaking) in Bu-Ac.

Spectrophotometry. The photometric apparatus employed for estimation of chromatographically separated amino acids proved inadequate for densitometry of the yellow spots produced by the above procedure, and a Perkin-Elmer flame photometer was adapted for use as a transmittance spectrophotometer. A 500-watt studio projection bulb and a 100-ml. round-bottomed flask filled with 1% aqueous copper sulfate were clamped to a tall ring stand beside the photometer in such a manner as to focus a beam of light through a 5-mm. hole in the center of a mask fitted to the chimney opening of the instrument. A concave microscope mirror was mounted under the hole of the mask and in front of the instrument's light entry slit by means of a one-hole rubber stopper wedged into the light trap opposite the slit, and the mirror was adjusted to direct into the slit a portion of the light scattered by filter paper chromatograms placed over the hole in the mask. To hold the paper flat against the hole in the mask while permitting its movement horizontally a petri dish with a flat-molded bottom was held in the light beam and pressed gently against the top of the paper by means of a three-pronged wire attached to the ring stand. Ordinarily the instrument was set at 440 mμ, adjusted to give a meter reading of 100 through a sprayed but spot-free background area of a chromatogram, and each visible spot then surveyed to find the area yielding the lowest meter reading, which was recorded as its per cent transmittance or converted to absorbance ($A = \log 1/T$). The background setting was readjusted occasionally to correct for drift and line voltage fluctuations, and duplicate readings of a given spot rarely differed by more than 5%.

No provision was made for repeatedly moving the paper between two precisely reproducible positions, but it was possible to determine the absorption spectra of chromatographic spots rapidly and with moderate accuracy by first recording the meter readings for a series of wave-length settings with a background area of a chromatogram in the light beam, then repeating the process with a colored spot in the beam, and expressing the latter set of data as percentages of the corresponding background readings. More precise measurements were made in some cases by mounting excised chromatographic spots and background areas in a cuvette holder for studying their light absorption in a Beckman Model DU spectrophotometer.

Syntheses. β-Ureidoisobutyric acid, melting point 120–121° C., was prepared by dissolving 0.01 mole of β-aminoisobutyric acid in 25 ml. of water, adding 0.02 mole of potassium cyanate, heating at 80° C. for 1 hour, cooling, stirring with 12 grams of Dowex 50-X (H-form) resin, filtering, evaporating, and recrystallizing from water. Several of the other ureido compounds needed for testing were not commercially available and were similarly prepared from the corresponding amino compounds.

RESULTS AND DISCUSSION

Separations. Listed in Table I are a number of compounds which react with *p*-dimethylaminobenzaldehyde to give yellow spots on paper chromatograms, together with their R_f values in three solvent systems. Most of these compounds were directly detectable by spraying with an acidified solution of *p*-dimethylaminobenzaldehyde, and even dihydropyrimidines occasionally showed a very weak reaction suggesting some acidic hydrolysis, but for sensitive detection of the dihydropyrimidines it was necessary first to spray the chromatogram with a solution of sodium hydroxide. Faint yellow spots were also observed occasionally in regions of the chromatogram containing a high concentration of amino acid or inorganic salt, and probably these cases represented a nonspecific effect on background color development.

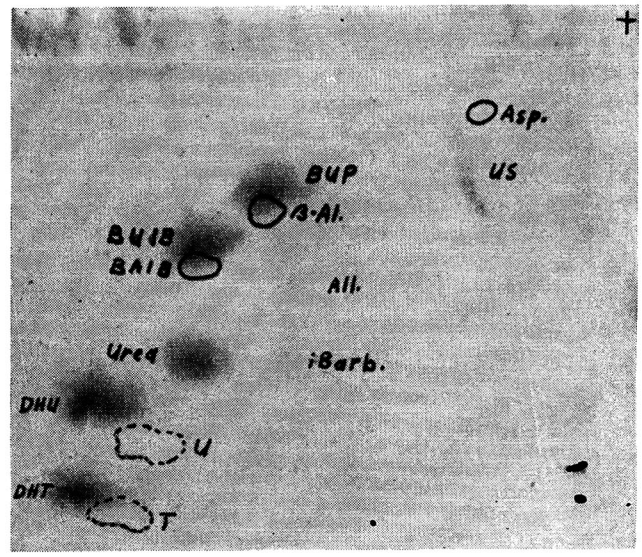

Figure 1. Two-dimensional chromatogram of complex mixture

T. Thymine
U. Uracil
BAIB. β-Aminoisobutyric acid
β-Al. β-Alanine
Asp. Aspartic acid
DHT. Dihydrothymine
DHU. Dihydrouracil
BUIB. β-Ureidoisobutyric acid
BUP. β-Ureidopropionic acid
US. Ureidosuccinic acid
All. Allantoin
iBarb. Isobarbituric acid
(The last three spots are just to left of the labels, but very faint.)

The ureido acid R_f values showed some variation, and occasionally the chromatographic spot from a single ureido acid had the appearance of two overlapping spots. This variability might be explained as a tendency toward salt formation with substances in the atmosphere, solvents, paper, or the mixture analyzed, because it was noted only with the acidic compounds studied.

In Figure 1 is shown a two-dimensional chromatogram of a complex mixture initially placed at the upper right corner and resolved consecutively with the phenol–water and *sec-* and *tert*-butyl alcohol–water solvents (Table I). The chromatogram was first examined under an ultraviolet lamp (2537 A.) for the marking of pyrimidine spots (dotted lines), treated with ninhydrin to show amino acids (solid lines), sprayed with alkali and with acidic *p*-dimethylaminobenzaldehyde (which destroyed most of the ninhydrin color), and finally photographed through a blue filter to show the yellow spots as dark areas. Dilution studies using the above procedure with the uracil series of compounds indicated that the minimum concentration for visual detection of uracil on chromatograms was about 10 mμ moles per sq. cm., whereas the color tests for dihydrouracil, β-ureido-

propionic acid, and β-alanine showed about 10 times that sensitivity.

Estimations. Spectrophotometric data from chromatograms treated with *p*-dimethylaminobenzaldehyde were rather variable, but showed that with adequate standardization this color reagent may be used for estimation of dihydropyrimidines and β-ureido acids on filter paper chromatograms. Typical calibration data collected over a period of several days are plotted in Figure 2, with each point representing data from a single one-dimensional chromatogram developed in a butyl alcohol–water mixture (Table I). The dihydrothymine solution was applied as a single 5-μl. droplet, and the solvent boundary was allowed to travel about 30 cm. beyond this point. The dotted line represents the equation fitted for least mean square deviation: $\log Q = 1.6 (1 - T)$ where Q is the quantity (millimicromoles) of dihydrothymine on the chromatogram, and T is the transmittance at 440 mμ through the densest portion of the yellow spot, with a sprayed but spot-free area of the paper taken as showing 100% transmittance. The standard deviation of the true log Q values from those predicted by the equation was ±0.1, indicating that in about two thirds of the chromatograms of a series such as this, the fitted curve could be used to estimate dihydrothymine concentration from a single spectrophotometer reading with an error of less than 25%.

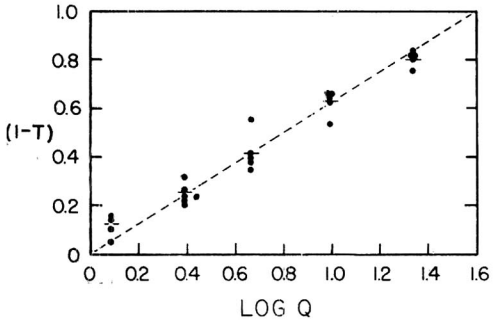

Figure 2. Relationship between light absorption at 440 mμ (1 − T) and number of millimicromoles of dihydrothymine present (Q) on one-dimensional chromatograms

Other series of standard chromatograms, prepared on the same days as the series represented by Figure 2, gave calibration curves (fitted by inspection) which appeared in some cases to be about the same as that shown and in others to be approximately parallel to it, but lower. For example, Figure 2 could apparently have been used without a significant correction factor for estimating the dihydrothymine concentration in 10-μl. droplets chromatographed as above, but gave estimates averaging 70% of the true values when the initial spot was further enlarged by using 25-μl. droplets. That is, the color reaction under the latter conditions was less intense (but apparently not less reproducible) and followed a calibration equation estimated as: $\log Q = 1.6 (1 - T) + 0.15$. Calibration curves for dihydrothymine were essentially the same as those for β-ureidoisobutyric acid, dihydrouracil, and β-ureidopropionic acid prepared under comparable conditions (indicating essentially complete alkaline hydrolysis of the dihydropyrimidines), whereas the test for urea under the standard conditions was only about 40% as sensitive, or $\log Q = 1.6 (1 - T) + 0.4$, and allantoin was still less chromogenic. On large two-dimensional chromatograms the spots had greater opportunity for diffusion, yielding a dihydrothymine calibration equation similar to that found for one-dimensional urea chromatograms, but with a somewhat greater scatter of individual points.

Experimental calibration curves such as that shown in Figure 2 tend to assume an S-shape, and this tendency could be accentuated by inclusion of higher and lower amounts of dihydrothymine. Analyses could be made at much higher levels if they were based on spot area or on attempts to estimate an average transmittance value for the whole spot, but such procedures tended to give highly variable results. Elution of chromatographic spots sometimes offers a tedious but theoretically excellent means of bypassing spectrophotometry on paper (with its inherent difficulties due to variations in spot sizes, shapes, transmittance gradients, etc.), but studies of the nature of the β-ureido acid color reaction make the development of a convenient, sensitive, and accurate elution procedure seem improbable in this case (*3*).

In most biological experiments the limited number of major *p*-dimethylaminobenzaldehyde-reactive compounds—e.g., dihydrothymine, urea, and β-ureidoisobutyric acid—have appeared to be adequately separated from interfering substances by simple one-dimensional chromatography such as that described for Figure 2 and shown as the vertical separations in Figure 1. In such cases the biological mixture could be applied along one edge of a filter paper sheet as a row of droplets interspersed with droplets from standard solutions containing higher and lower concentrations of the compounds to be estimated. The whole group of spots could then be developed together chromatographically and colorimetrically to yield, with comparative ease, estimations showing an error of ±20% or less (depending on the number of replicate chromatograms prepared). The spot to be measured occasionally overlapped or was distorted by an interfering substance, however—e.g., a small dihydrouracil spot and a large urea spot—requiring special treatment such as two-dimensional chromatography before even a very rough estimation was possible. Since conditions can be kept more nearly identical for replicate one-dimensional chromatograms than for the more difficultly prepared two-dimensional variety, the achievement of a given degree of accuracy ordinarily requires a somewhat greater number of chromatograms and a considerably greater expenditure of time and effort when one-dimensional separations are inadequate.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Batt, R. D., Martin, J. K., Ploeser, J. McT., and Murray, J., *J. Am. Chem. Soc.* **76**, 3663 (1954).
(2) Berry, H. K., Sutton, H. E., Cain, L., and Berry, J. S., University of Texas, Austin, Tex., Publ. **5109**, 22 (1951).
(3) Cline, R. E., and Fink, R. M., ANAL. CHEM. **28**, 47 (1956).
(4) Dent, C. E., *Biochem. J. (London)* **43**, 169 (1948).
(5) Ehrlich, P., and Boehme, A., *Centr. Bakterial. Parasitenk* **40**, 129 (1905).
(6) Fink, K., Henderson, R. B., and Fink, R. M., *J. Biol. Chem.* **197**, 441 (1952).
(7) Fink, K., Henderson, R. B., and Fink, R. M., *Proc. Soc. Exptl. Biol. Med.* **78**, 135 (1951).
(8) Fink, K., and McGaughey, C., *Federation Proc.* **13**, 207 (1954).
(9) Fink, R. M., Cline, R. E., and Koch, H. M. G., *Ibid.*, **13**, 207 (1954).
(10) Fink, R. M., Fink, K., and Henderson, R. B., *J. Biol. Chem.* **201**, 349 (1953).
(11) Funk, C., Merritt, A. J., and Ehrlich, A., *Arch. Biochem. and Biophys.* **35**, 468 (1952).
(12) Watt, G. W., and Crisp, J. D., ANAL. CHEM. **26**, 452 (1954).

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Separation of Three Mono-*O*-Methyl-D-Glucoses

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This study was undertaken to develop a rapid, simplified procedure for quantitatively separating the three mono-*O*-methyl-D-glucoses. Present techniques are laborious and unsatisfactory. This paper describes a satisfactory method for quantitative separation of these isomers by paper chromatography.

INVESTIGATIONS on the distribution of methoxyl groups in partially methylated cellulose or starch samples would be greatly facilitated by a satisfactory procedure for the separation of the isomers of both the mono- and di-*O*-methyl-D-glucoses which may be obtained by hydrolyzing these substituted polysaccharides (*7, 9, 10*). The use of paper chromatography for analyzing mixtures of the three possible mono-*O*-methyl-D-glucoses obtainable—i.e., 2-*O*-methyl-D-glucose, 3-*O*-methyl-D-glucose, and 6-*O*-methyl-D-glucose—is described herein. To date, the only known method for analyzing such mixtures involves the oxidation of these sugars by periodic acid, followed by alkaline hydrolysis and the separation and identification of the degradation products by paper chromatography (*5*). Unfortunately, this procedure cannot be applied quantitatively.

Table I. Chromatographic Separation of Two Known Mixtures of Three Mono-*O*-Methyl-D-glucoses

Sugars in Mixture	Mixture I			Mixture II		
	Amount spotted, mg.	Amount observed, mg.	% error	Amount spotted, mg.	Amount observed, mg.	% error
Glucose	1.45	1.38	4.8	1.08	1.14	5.6
2-*O*-methyl-D-glucose	0.66	0.69	4.6	0.90	0.84	6.7
3-*O*-methyl-D-glucose	0.54	0.60	11.1	0.90	0.89	1.1
6-*O*-methyl-D-glucose	0.47	0.48	2.1	0.63	0.65	3.2
Total	3.12	3.15		3.51	3.52	

On the other hand, the separation of mixtures of mono-, di-, and trisubstituted methylglucoses from each other has been accomplished by several methods, including vacuum distillation (*8, 11*), extraction (*1, 6*), and paper chromatography (*3, 4, 9*). The latter method has been applied quantitatively in this and other laboratories with errors of less than 5%.

Because of the lack of a direct method for separating the isomers of the mono-*O*-methyl derivatives, investigators in the past have been forced to resort to a series of time-consuming substitution and degradation reactions in determining the relative distributions of methoxyl groups in partially methylated celluloses (*7, 10*). These reactions include: tosylation and iodination of the polymer for determining unsubstituted primary hydroxyl groups; hydrolysis to simple sugars and oxidation of their *cis*-1,2-glycol groups with lead tetraacetate to determine the amount of unsubstituted hydroxyls in the 2- position; periodic acid oxidation of the *trans*-2,3-glycol groups in the polymer and of the *trans*-2,3- and 3,4-glycol groups in the glucosides obtained on methanolysis to establish the number of unsubstituted hydroxyls in the 3- position; and finally fermentation of glucose in the hydrolyzate to give the number of unsubstituted anhydroglucose units in the polymer. While these reactions are all fairly well characterized, their application on a quantitative basis offers some obvious difficulties and has never been proved to be completely justified. The use of quantitative paper chromatography eliminates these uncertainties and considerably reduces the amount of time required for such an analysis.

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PROCEDURE

Unidimensional, descending chromatograms were run continuously at 30° C. using sheets of Whatman No. 1 filter paper cut in 6 × 22 inch strips and serrated at the lower edges. Three sugar mixtures were spotted on each sheet 1.5 inches apart and 3 inches from the top. To sharpen the spots during developing, the strips were removed from the chamber every 15 hours and air dried in the hood.

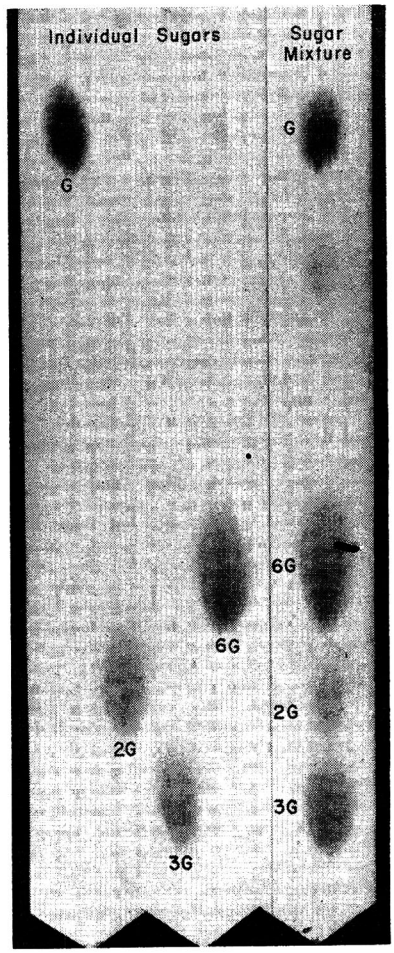

Figure 1. Unidimensional chromatogram of glucose and three mono-*O*-methyl-D-glucoses

G. Glucose
2*G*. 2-*O*-methyl-D-glucose
3*G*. 3-*O*-methyl-D-glucose
6*G*. 6-*O*-methyl-D-glucose

The chromatograms were developed with the top layer of a 2 to 5 to 5 mixture of 2,4,6-collidine–ethyl acetate–water. The solvent system was passed down the paper for 65 hours, excluding the drying periods. The sugar spots were located by spraying a guide strip cut from each sheet with a 3% solution of *p*-anisidine hydrochloride in 1-butanol and heating in an oven at 100° C. for 10 minutes (*3*). A typical separation obtained by this procedure is shown in Figure 1.

Quantitative determinations were made according to the method of Hirst (*2*) by elution, oxidation of the reducing sugars in the eluent with a buffered iodine solution, regeneration of un-

reacted iodine, and titration with 0.1*N* thiosulfate using a Gilmont ultramicroburet of 1-ml. capacity.

RESULTS

Two known mixtures of glucose and the three pertinent mono-*O*-methyl-D-glucoses were analyzed. A comparison of the amount of each sugar weighed into the mixture and spotted on the chromatogram to the amount of that sugar determined by elution and titration is presented in Table I. It is evident from this table that a quantitative separation of the mono-*O*-methyl-D-glucoses was obtained. An indication of the efficiency of the separations is given in the per cent error column.

The colors of the spots of the three mono-*O*-methyl-D-glucoses were not identical: 2-*O*-methyl-D-glucose gave a lavender spot, whereas the 3-*O*-methyl-D-glucose spot was yellowish brown and the 6-*O*-methyl-D-glucose spot a brownish yellow.

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LITERATURE CITED

(1) Brown, F., and Jones, J. K. N., *J. Chem. Soc.* **1947**, p. 1344.
(2) Hirst, E. L., Hough, L., and Jones, J. K. N., *Ibid.*, **1949**, p. 928.
(3) Hough, L., Jones, J. K. N., and Wadman, W. H., *Ibid.*, **1950**, p. 1702.
(4) Jones, J. K. N., and Smith, F., *Advances in Carbohydrate Chem.* **4**, 243 (1949).
(5) Lemieux, R. U., and Bauer, H. F., *Can. J. Chem.* **31**, 814 (1953).
(6) Macdonald, J. T., *J. Am. Chem. Soc.* **57**, 771 (1935).
(7) Mahoney, J. F., and Purves, C. B., *Ibid.*, **64**, 9, 15 (1942).
(8) Peat, S., and Whetstone, C., *J. Chem. Soc.* **1940**, p. 276.
(9) Rebenfeld, L., and Pacsu, E., *Textile Research J.* **24**, 941 (1954).
(10) Timell, T., "Studies on Cellulose Reactions," Esselte Aktiebolag, Stockholm, Sweden, 1950.
(11) Traube, W., Piwonka, R., and Funk, A., *Ber. deut. chem. Ges.* **69B**, 1483 (1936).

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Chromatographic Determination of Gum in Fuels

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A novel chromatographic method for the determination of gum in small fuel samples is presented. The gum content is related to the length of a brown zone observed when the sample is displaced over silica gel in a very small column with 1-methylnaphthalene as solvent and acetone as eluent. Although the method was developed for use in research on fuels of limited availability, its simplicity makes it more generally attractive. This chromatographic determination of gum appears to be applicable to soluble and insoluble gum in jet fuels, gas oils, and possibly gasolines. Additional data will be required to establish its merits for routine control purposes.

FUEL deterioration during storage in the presence of air results in the formation of various oxidized products, which leave a gummy brown residue on evaporation. Often precipitation of insoluble material also occurs. Prevention or minimization of gum formation is a continuing problem which is particularly severe with cracked fuels. The problem is becoming more acute as our dependence on cracked fuels increases and the engines in which the fuels are consumed become more complex and more critical.

Gum is normally determined by evaporation of a 50-ml. sample of fuel at elevated temperatures in a jet of air or steam. The residue is weighed and the gum content is reported in milligrams per deciliter (*1*). This method is time-consuming, requires special equipment and relatively large samples, and sometimes gives inconsistent results. Also, the results are affected by the end point of the fuel (*4*). The volume requirement is not significant normally, but becomes important in studies on experimental or salvaged fuels of limited availability.

In connection with a study of stability of experimental fuels, adsorption on silica gel was employed as a means of isolating the gum fraction. During the elution step it was noted that a considerable brown zone developed. On the basis of this observation an investigation was made to see if the length of the dark zone could be related to the gum content. As fuel volumes frequently were limited, these studies were made in equipment similar to that used for small scale chromatography as applied to hydrocarbon group analysis (*2*). This paper introduces the resulting chromatographic or "chromatogum" method which has been found suitable for a wide variety of jet fuels, as well as for gas oils. Incomplete data suggest that it may also be applied to gasoline.

To meet the needs of the above stability study it was necessary to show a correlation between the chromatographic and the steam-jet gum results for jet fuels and gas oils having a wide range of gum contents. For routine use on unaged samples more emphasis on the low gum region is desirable.

In the long view, it may be preferable to use the chromatographic method as a new approach, independent of the evaporation methods, for determining oxidized material in fuels.

SUMMARY OF METHOD

A small glass adsorption column with a long capillary extension is packed with fine activated silica gel and 0.5 to 1 ml. of sample is introduced. This is followed by a small quantity of 1-methylnaphthalene and then acetone eluent. The length of a brown zone containing gum is measured and empirically correlated with the steam-jet gum content.

DEVELOPMENT OF METHOD

A typical jet fuel sample was divided and one portion was aged with oxygen at elevated temperature to increase its gum content. These samples were subjected to chromatography to see whether zones were obtained that could be related to the gum contents. When a sample was added to a long, narrow column of Davison's Grade 923 (100 to 200 mesh) silica gel and displaced by isopropyl alcohol, a brown zone was formed adjacent to the alcohol front. This zone was unexpectedly long for the small amount of gum present, and its length was approximately proportional to the gum content. Thus the feasibility of the chromatographic approach was indicated. Additional samples of jet fuels, as well as gasolines and gas oils, covering the range of gum concentrations of

interest, were used in the study of the variables affecting the analysis.

Eluents. Eluents investigated include isopropylamine, dimethylformamide, isopropyl alcohol, methanol, pyridine, formic acid, and acetone. None of these displaced all of the brown material from all of the samples. However, acetone, isopropylamine, and methanol were among the best and were about equally good. Acetone was selected as the eluent for future work for reasons of convenience.

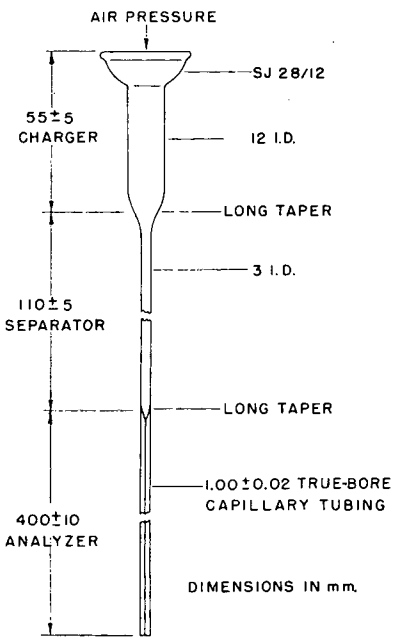

Figure 1. Adsorption column

Column Design. A special column, designed to provide sharp brown zones with very small samples, is shown in Figure 1. It consists of a relatively broad "charger" section at the top, a narrower "separator" section in the middle, and a true-bore capillary "analyzer" section at the bottom in which the zone measurements are made.

Sample Size. The small gum column requires only 0.5 to 1.0 ml. of jet fuels or gas oils. For greater accuracy in the low gum region, and particularly for gasolines (which normally exhibit very low chromatographic gum contents), it may be necessary to employ up to 5 ml. of sample. This requires a preliminary, rapid gum separation in a second column. The data in this report, however, were all obtained by the one-column technique.

Flow Rate. The brown zone becomes longer and more diffuse as the pressure and flow rate are increased. It was found that pressures of up to 10 pounds per square inch can be employed when the brown zone is in the upper part of the column, but that when it reaches the lower part, where readings are taken, the pressure should be decreased to 2 or 3 pounds per square inch.

Inhibitors. Ionol (2,6-di-*tert*-butyl-4-methylphenol) and Tenamine-1 (*N*-*n*-butyl-*p*-aminophenol) in abnormally high concentration (0.03% or 10 pounds per 5000 gallons) had little effect on the brown zone obtained with an aged thermally cracked jet fuel. With the same amount of UOP-5 (*N*,*N*-di-*sec*-butyl-*p*-phenylenediamine) there was a 10 to 15% increase in gum zone length. It is therefore concluded that the inhibitors in practical concentrations (1 pound per 5000 gallons) would not affect the results significantly.

Adsorbents and Sample Composition. The relatively long brown zones obtained on Grade 923 silica gel with samples containing at most, only a fraction of a per cent of gum, may be explained on two bases: The gum molecules are too large to enter the pores of the adsorbent (32 A. average pore diameter); and the gum forms an adsorption azeotrope or "asorbotrope" with some of the sample aromatics. This asorbotrope, or mixture which is not separated by passage over the adsorbent (*3*), is more strongly adsorbed than the remaining sample aromatics and, hence, appears between them and the acetone front. The brown zone is long, because the concentration of gum in the asorbotrope is low. These hypotheses have been studied and both have some merit.

Several adsorbents with larger pores than the Grade 923 silica were employed for gum separation, including Grade 70 silica gel (100 A.), alumina (40 A.), and silicic acid (50 to 100 A.). In all cases the gum was much more strongly adsorbed and in a shorter zone than on Grade 923; in fact the gum often could not be displaced by any available eluent. Adsorbents of smaller pore size were not studied. Since Grade 923 gel appeared to be suitable, it was used for all subsequent work.

Fuel composition also affects the length of the zone. For example, when benzene was added to certain jet fuels, or gas oils, the zone became much shorter. Gasoline gum zones are also very short, because perhaps of the presence of benzene or toluene. These observations suggested that an asorbotrope of gum with aromatics (which, like the gum, are adjacent to the eluent front) is formed. Apparently the length of the brown zone depends on the concentration of gum in the asorbotrope, which in turn depends on the nature of the aromatics present. Hence a search was made for a single-component solvent that would displace the multicomponent, unpredictable aromatics.

Asorbotroping Solvents. In order to be suitable for chromatographic gum determination the solvent must meet two adsorbability requirements. First, it must displace the sample aromatics, but not the gum. Thus both solvent and gum appear as an asorbotrope just below the eluent front. Second, the solvent must form a reasonably long brown zone for a given gum content.

It was found that anisole, ethylene dichloride, isopropyl chloride and 1-methylnaphthalene are in the right adsorbability range. Of these, 1-methylnaphthalene forms the longest brown zones and hence provides the greatest precision. Enough 1-methylnaphthalene (0.1 ml.) is employed to form an asorbotrope with all of the gum, the excess 1-methylnaphthalene being displaced by the asorbotrope. The 1-methylnaphthalene reduced the very long gum zones obtained with certain gas oils and increased the zones obtained with gasolines, but had little effect on jet fuels. Thus all fuels were put on a common chromatographic basis. Since the composition of jet fuel is not rigidly specified, it is considered preferable to employ the solvent for this, as well as for other types of fuel.

Dilution of Benzene-Free Fuels. When gas oils were analyzed without dilution, the flow through the column was so slow that 5 or 6 hours were required for the analysis. In addition the gum was developed downward during the sample addition and in extreme cases it had entered the analyzer before the 1-methylnaphthalene was added. Dilution of the sample with about 50% of its volume of benzene corrected both of these difficulties. The benzene is displaced by the 1-methylnaphthalene, which is added after the sample. Dilution of gas oils and other benzene-free fuels—for example, Diesel fuel or gasoline saturates—is recommended.

Use of Dye for Light-Colored Fuels. Fuels containing very little gum are so light in color that the brown zone is barely perceptible. In such cases a red dye may be added to intensify the color. This should not be adopted as a routine measure because, if the brown zone is very long, the dye may not color the entire zone, but only the upper part of it. The dyed portion could then

be mistaken for the entire gum zone. The dye is also used when determining the "blank" brown zone due to the 1-methylnaphthalene solvent added.

Purification of 1-Methylnaphthalene. The Eastman "practical" grade 1-methylnaphthalene is very dark, but can be adequately decolorized chromatographically as described in the section on analytical procedure. All data in this paper were obtained with 1-methylnaphthalene, yielding a blank brown zone about 15 mm. long in the presence of the red dye.

ANALYTICAL PROCEDURE

The 1-methylnaphthalene is decolorized by passing it over about half its volume of silica gel with acetone as eluent. The purified material should have a blank not exceeding 15 mm. in the presence of the indicator dye, ethyl red. The indicator is conveniently employed in the form of dyed gel, made by slurrying 0.10 gram of dye and 100 grams of gel with methanol, and then evaporating the solvent at room temperature in a stream of air with occasional stirring until the dyed gel is free-flowing.

The end of the column is plugged with cotton and packed with silica gel to a point 30 to 40 mm. below the top of the separator section. If the sample is light colored, or when the blank is to be determined, 10 mm. of dyed gel is added.

Soluble and insoluble gums require somewhat different techniques.

For soluble gum in jet fuels or gas oils 0.50 ml. of sample is introduced, except when the gum content is below about 30 mg. per dl. in which case 1.00 ml. is added. However, any convenient sample size in this range can be used. If the benzene plus toluene content is not known, or is less than 10%, the sample should be preceded by about half its volume of benzene. The sample is added immediately to promote mixing with the benzene before the latter is taken up by the gel.

For the determination of soluble gum in low-gum fuels, including many gasolines, a 5-ml. sample may be required. Then, to save time, a preliminary rapid gum separation is made on a column of 5 mm. inside diameter by 150- or 200-mm. length. The sample (diluted with benzene if necessary) is followed by isopropyl chloride, which displaces the hydrocarbons, and the gum is eluted with acetone and collected in a small flask. The eluent is removed at room temperature in a stream of air, and the gum is dissolved in chloroform and transferred to the gum column. No benzene diluent is necessary here.

A pressure of 3 pounds per square inch is applied until the liquid front reaches the middle of the analyzer section, when the pressure may be increased to 5 or 10 pounds per square inch until the sample is taken up. Then 0.10 ± 0.02 ml. of purified 1-methylnaphthalene is added, the walls are rinsed with a few drops of acetone, and the charger is filled with acetone. Pressure is again applied at 3 pounds per square inch (2 pounds per square inch for gasoline) until the brown zone has progressed well into the analyzer.

The length of the brown zone is measured against a white background. It is the distance between the highest point of strong brown or yellow color and the lowest point where a color change is still apparent. Usually the column is white below the brown zone, but it may be uniformly yellow if the sample contains high boiling colored aromatics. The reading is repeated at 20- to 30-mm. intervals until the zone length is stable. Readings should not be taken when the acetone front is within 80 to 100 mm. of the bottom of the column, as the zone length tends to increase somewhat in this region. Occasionally the brown zone becomes severely skewed in the separator and does not reach equilibrium in the analyzer. This can be corrected by employing a lower pressure after the acetone is added and until the gum zone enters the analyzer, and then reverting to the specified pressure.

Insoluble gum, by definition, is the precipitate retained on a "fine" (Pyrex brand) sintered-glass filter after washing with *n*-hexane. The gum thus isolated, is dissolved in a polar solvent and sampled for the chromatographic determination. If chloroform is employed as a solvent, the procedure is the same as for soluble gum. When the commonly used, strongly polar solvents such as acetone or ethyl acetate are employed, these must be removed, and the gum redissolved in chloroform. No benzene diluent is necessary.

The column may be cleaned by rinsing out the gel with a jet of water from a hypodermic tubing, or, it may be left under pressure until dry, inverted, and the gel removed by tapping gently.

The brown zone length may be empirically converted to milligrams of gum by reference to graphs such as shown in Figure 2. From the equations for the lines the gum contents may be calculated in milligrams per deciliter as follows:

For JP-4 jet fuels

$$\text{Gum, mg./dl.} = \frac{0.61}{V}(L - B)$$

For gas oils

$$\text{Gum, mg./dl.} = \frac{0.54}{V}(L - B)$$

where V = volume of sample, ml., L = length of brown zone, mm., and B = 1-methylnaphthalene blank, in mm.

Similar expressions may be derived for other fuels as data become available.

DISCUSSION OF RESULTS ON JET FUELS AND GAS OILS

The jet fuels tested represent straight-run, thermally cracked, catalytically cracked, and blended samples from various sources. This selection is considered to be a good cross section of emergency-type JP-4 jet fuels. Most of the gas oils consist of a catalytically cracked stock having a 90% point of 585° F. (310° C.) and 40° F. distillation fractions of this material. It is believed that other gas oils in this boiling range would behave similarly.

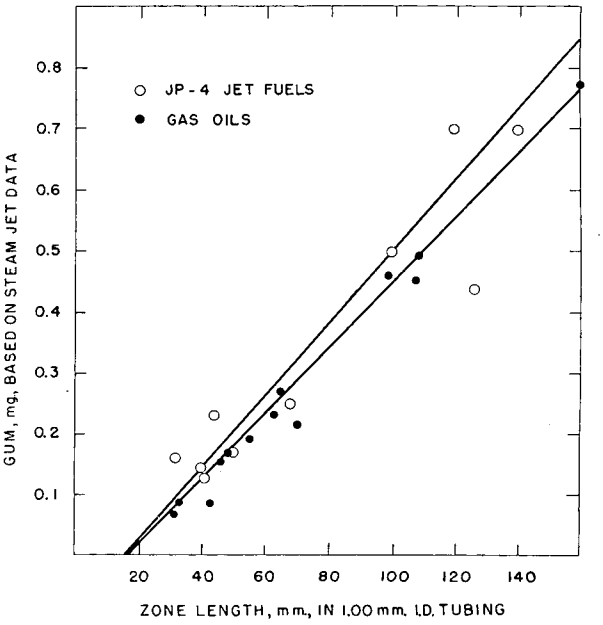

Figure 2. Chromatographic determination of soluble gum

Soluble Gum. The soluble gum contents of these fuels were determined by a steam-jet evaporation procedure (*1*, at 500° F.). The chromatographic gum values were then determined as described. The JP-4 jet fuel and gas oil results are presented in Figure 2, in which the actual weight of gum in the sample is plotted against the brown zone length. Thus the plots are independent of sample size. Two lines have been drawn, one for each type of fuel, because it is statistically probably that the lines should be separate. However, more data would be required to prove this rigorously. The scatter of the points may

be attributed to the limited repeatability of both steam-jet and chromatographic methods. The standard deviation of the repeatability was about 7% in both cases, and the standard deviation between the steam jet and chromatographic methods was about 14%.

The jet fuels of Figure 2 were aged in the desert. A few additional samples that had been subjected to accelerated aging were studied, and the data indicated that the steam jet–chromatographic correlation for jet fuels is valid up to an aging temperature of 100° C.

The gas oils were aged at 110° F. (43° C.) in a constant temperature room. Additional results from samples aged at 70° C. indicated that these could be treated by the same correlation. However, gas oils aged at 100° C. gave a much steeper curve, and hence the chromatographic method is suitable for only a rough determination on such samples.

Insoluble Gum. Available data are not conclusive, but it appears that Figure 2 can be used for both soluble and insoluble gum in jet fuels (JP-4) and gas oils. However, earlier work under different analytical conditions indicated that results from fractions of narrow boiling gas oil of 220 to 240 molecular weight and with high insoluble gum content may diverge considerably from the line drawn.

DISCUSSION OF PRELIMINARY RESULTS ON GASOLINE

Preliminary tests have indicated that there may be a correlation between the chromatographic brown zone and the steam-jet gum (500° F.) content for gasolines. This observation is interesting and encouraging. However, gasoline gums are normally determined by air-jet evaporation at 330° F. A study of the relation between the air-jet and chromatographic determinations of gum is in progress.

EVALUATION OF CHROMATOGRAPHIC METHOD

The chromatographic method for determination of gum gives values that appear to be acceptably close to the steam-jet results for soluble and insoluble gum in the jet fuels and gas oils tested. The reliability is uncertain in the case of gas oils aged above 70° C. and of insoluble gum from severely aged gas oils of high molecular weight. Only about 1 ml. of sample is required, and one operator with facilities for 10 columns can make 15 to 30 determinations in 8 hours. Preliminary data indicate that the method may be applicable to gasoline.

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LITERATURE CITED

(1) Am. Soc. Testing Materials, Philadelphia, Pa., "ASTM Standards on Petroleum Products and Lubricants," D 381-54T, 1954.
(2) Criddle, D. W., and Le Tourneau, R. L., ANAL. CHEM. **23**, 1620 (1951).
(3) Knight, H. S., and Groennings, S., *Ibid.*, **26**, 1549 (1954).
(4) Nixon, A. C., Cole, C. A., and Walters, E. L., "Factors in Jet Fuel Stability. Occurrence and Measurement of Gums in Jet Fuel," unpublished.

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Determination of Specific Activity of Carbon-14–Labeled Sugars on Paper Chromatograms Using an Automatic Scanning Device

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A simple method was needed for determining the quantitative distribution of carbon-14–labeled sugars and sugar derivatives in solutions resulting from biological studies. The paper chromatographic separation of these carbon-14 substances and the localization of active sites on the chromatogram were possible by scanning the developed chromatographic strip with an automatic scanning device. This device is applied to the quantitative determination of the specific activity of carbon-14–labeled sugars by two methods. One method employs a summation equation involving the areas under the scanning curves and the total specific activity of the solution being assayed. The second uses known carbon-14–labeled sugar solutions which serve as standards. The heights of the scanning curves of the standards were measured and a straight-line standard curve was prepared. The heights of the scanning curves of the unknowns were measured also, and their specific activities calculated from the standard curve. Determination of the specific activity of carbon-14–labeled lactose, galactose, and glucose by either method results in an error no greater than 3%.

THE distribution of radioactivity among a number of labeled components in a solution or mixture can be of considerable importance to an investigator. The application of paper chromatography for the separation of radioactive components and their subsequent localization by autoradiographic techniques is well known. The autoradiographic technique has, in the past, suffered from being time-consuming and/or inefficient, especially when dealing with weak beta emitters of low specific activity. The scanning of a paper strip by a systematic manual exploration with a Geiger counter is also, to a certain extent, inefficient and tedious.

The difficulties encountered with autoradiography and hand scanning have been offset by the construction of devices for automatically scanning paper radiochromatograms (*2, 3, 5, 7, 9, 10, 12–14*). Each consists of a mechanism for moving a paper strip past a collimating slit in front of a Geiger counter or ionization chamber and an apparatus for recording the counting rate as a function of the position of the paper.

Two methods of using the automatic scanning device for the quantitative determination of the specific activity of carbon-14–labeled sugars after separation on paper chromatograms are reported herein. The first method is based on the determination of the specific activity of the unknown solution (two or

more carbon-14 sugars) prior to the determination of the specific activity of the individual components. The second method permits the determination of the specific activity of the individual carbon-14 sugars in solution using known standards without referring to the total specific activity of the sample being analyzed.

Table I. Calculation of Specific Activity Using Summation Equation

	Area[a]				Calcd. Specific Activity (1×10^{-4} μc./μl.)		
	Lactose	Galactose	Glucose	Total	Lactose	Galactose	Glucose
Activity placed on paper (μc. $\times 10^{-4}$)	53.9	40.2	38.2	132.3			
Volume (μl.) placed on paper (2-μl. applications)	6	4	20	30			
No. 1 scan	184	136	137	457	8.88	9.85	1.98
No. 2 scan	191	142	131	484	9.08	10.12	1.86
No. 3 scan	195	145	130	470	9.15	10.20	1.83
Av. calcd. specific activity					9.04	10.06	1.89
Theoretical specific activity					8.98	10.03	1.91
% dev. from theory					+0.7	+0.1	−1.0

[a] Count rate setting: 1000 c.p.m., slit width: 2.0 mm., Lasico planimeter no. 123 setting: 126.7.

SCANNING APPARATUS

The scanner used in this study (purchased from the Aquebogue Machine and Repair Shop, Aquebogue, Long Island, N. Y.) was designed by R. C. Anderson (*1*). Briefly, the scanner consists of a housing chamber with provisions for inserting a 0.5-, 1.0-, 1.5-, or 2.0-mm. window slot and a drum having a circumference of 35.8 cm., onto which the paper strip is attached (Figure 1). The dimensions of the housing chamber restrict the width of the strip to a maximum of 3.5 cm. Mounting the radiochromatogram on the revolving drum offers a number of advantages. First, there is no chance that the paper may be moved to one side or the other, because the radiochromatogram itself is "permanently" mounted. Thus, the possibility of a recording which does not represent a true picture of the radioactivity is eliminated. Another advantage, and one of convenience, is that the cycle can be repeated without the radiochromatogram being rethreaded. In addition, increased sensitivity is obtained by having the radiochromatogram mounted within the detector, as there is no loss of activity encountered by the air space from paper to detector, nor any loss due to the window thickness of the detector.

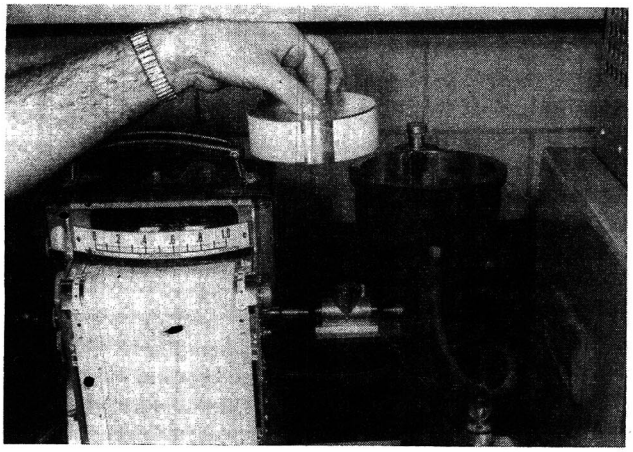

Figure 1. Chromatographic strip positioned on drum

Strip is being introduced into detecting chamber, after which chamber is sealed with a glass plate. Esterline-Angus recorder and detecting chamber are connected by a Metron variable speed-ratio-changer.

An Esterline-Angus recorder (Figure 2) drives the drum to which it is geared. A variable speed-ratio-changer (Metron) is available for adjusting the drum speed in relation to the chart speed. The detector is connected to a scaler (Atomic Instrument Co.), which in turn is connected to a precision ratemeter (Tracerlab, Inc.) and a linear nonoverloading amplifier and a 5000-volt power supply whose circuit was altered to that described by Van Slyke (*11*). The instrument is operated in the proportional region at 3750 volts employing methane as the counting gas. (A gas mixture consisting of 90% argon plus 10% methane is now being employed at an operating voltage of 2400 volts.)

A typical scan permitting the localization of activity is shown in Figure 3. Galactose-1-carbon-14 (courtesy of H. S. Isbell, National Bureau of Standards) was applied to a strip of Schleicher and Schuell 589 White Ribbon filter paper in 2-μl. applications. The curves represent 2.4×10^{-4}, 4.7×10^{-4}, 5.9×10^{-4}, 7.1×10^{-4}, and 11.8×10^{-4} μc., respectively. Measuring the area under each curve with a Lasico planimeter No. 123 (E. Dietzgen Co.) and plotting it against the activity yielded a linear relationship.

Figure 4 illustrates the linear relationship between the area under the scanning curves and the corresponding activities. It does not, however, serve as a reference standard curve. The procurement of an all-inclusive standard curve relating reproducible areas to known activities to be used in the determination of unknown activities was not attempted in this study. If the establishment of such a curve was possible, it would require the rigid control of such conditions as the chromatographic development procedure, the drying time of the filter paper prior to scanning, the absorption of the beta particles by the filter paper, the efficiency of the detector and the scaler, the differences in the chart and drum speeds, and the fluctuations in curve design due to the slippage of the connecting gears.

The sensitivity of this scanning device is well illustrated by the scanning record depicted in Figure 3. Comparable activities required 30 days of exposure to Eastman Kodak No-screen x-ray film in order to produce spots of sufficient intensity to be measured using a densitometer (Figure 5). Increasing the activity tenfold permitted autoradiographic detection in 3 days, which is still far more time-consuming than the average time of 1 hour generally devoted to scanning a strip.

DETERMINATION OF SPECIFIC ACTIVITY USING A SUMMATION EQUATION

The linear relationship between activity and the area under the scanning curve served as the basis for a quantitative measurement of the activities of the carbon-14–labeled sugars in solution. Experimental data showed that the areas under the curves were related to the activities, regardless of the initial area these solutions covered on the filter paper before and after chromatography. In other words, 2.4×10^{-4} μc. of galactose-1-carbon-14 placed on the paper as a 2- or 5-μl. spot still yielded comparable scanning areas.

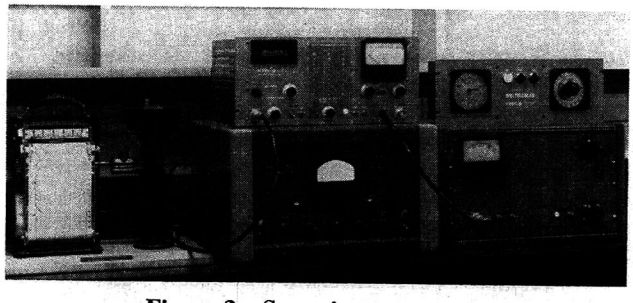

Figure 2. Scanning apparatus

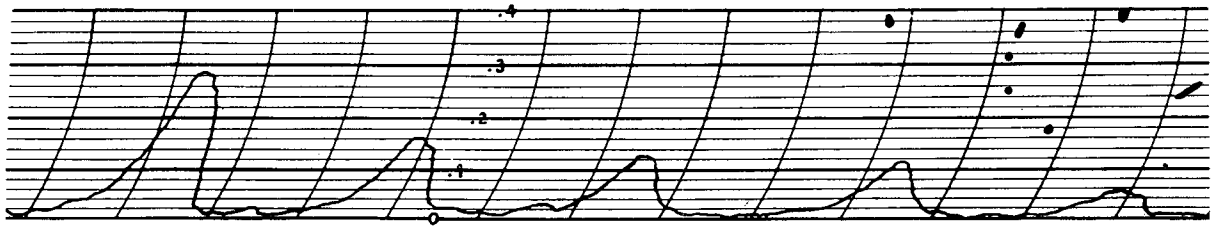

Figure 3. Typical scanning record

Source, galactose-1-carbon-14
Count rate setting, 1000 counts per minute
Slit width, 2.0 mm.
Area measured with No. 123 Lasico planimeter, 126.7 setting

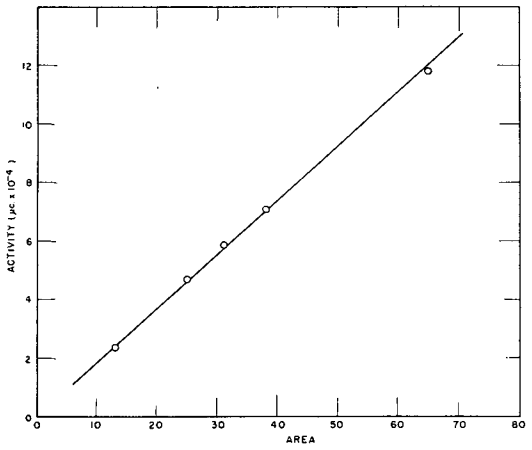

Figure 4. Relationship of activity to area under curves

Source, galactose-1-carbon-14
Count rate setting, 1000 counts per minute
Slit width, 2.0 mm.
Area measured with No. 123 Lasico planimeter, 126.7 setting

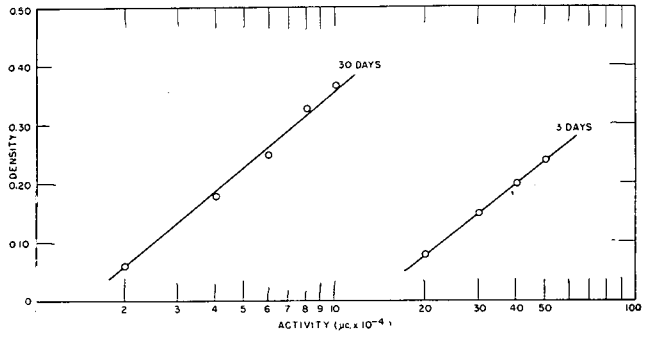

Figure 5. Variation of density with exposure time to Eastman Kodak no-screen x-ray film

Source, galactose-1-carbon-14

Experimental. This method using a summation equation consisted of separating the sugars (carbon-14–labeled lactose, galactose, and glucose) on Schleicher and Schuell 589 White Ribbon filter paper, using the solvent system 2.5 parts of ethyl acetate, 1.0 part of pyridine, and 3.5 parts of water. The chromatographic apparatus and technique for the separation of these sugars have been described (*6*).

After solvent development and thorough drying, which lessens the absorption effects of the residual solvent, a strip 3.5 cm. wide and 33 cm. long containing the separated sugars was cut, taped to the revolving drum, and then scanned using a 2.0-mm. slit width. This slit width gave maximum sensitivity of the four slits available (Figure 6).

The areas under the scanning curves were then measured using a planimeter and the specific activities of the individual sugars were computed using the equation:

$$X = \frac{A_s}{A_t}\left[\frac{C}{V}\right]$$

where X equals the specific activity (microcuries per microliter) of the carbon-14 sugar(s), A_s equals the area under the scanning curve of the labeled sugar, A_t equals the sum of the areas under all the curves, C equals the total activity (microcurie) placed on the paper, and V equals the volume (microliters) of solution placed on the paper.

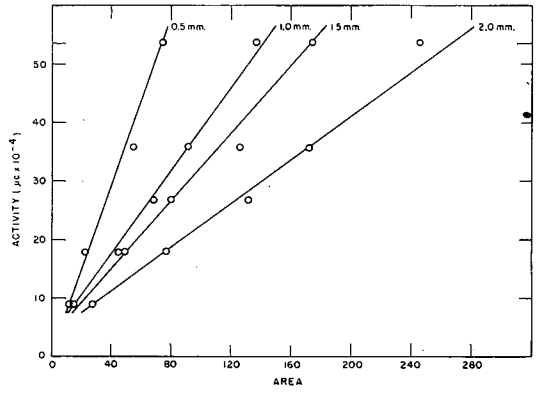

Figure 6. Variation of area of curves with slit width used in counting chamber of scanner

Source, lactose-1-carbon-14
Count rate setting, 1000 counts per minute
Area measured with No. 123 Lasico planimeter, 126.7 setting

Results and Discussion. Table I shows some typical results and errors involved in determining the specific activity of lactose-1-carbon-14, galactose-1-carbon-14, and glucose-1-carbon-14 in a solution containing the three sugars.

Where values for lactose, galactose, and glucose were desired, chromatographic separation of these components prior to scanning was essential. Normally a 16-hour development in the ethyl acetate–pyridine-water solvent system separated the three sugars sufficiently to permit the quantitative determination of their concentration by the direct photometric method (*6*). Such a development was not adequate when the specific activity was to be calculated, because the geometry involved during the counting of a radioactive spot made it imperative that sufficient separation be effected so that the scanning curve for each component start and finish at the base line of the recording chart. The values reported in Table I were obtained by scanning the strip containing the three sugars after separation in the ethyl acetate–pyridine-water (2.5:1.0:3.5) solvent system employing the multiple

descent technique (4), which included three separate 24-hour developments.

When three or more active components were present in a solution and sufficient chromatographic resolution to give individual curves without any overlapping was not possible, the specific activity of one separable component was obtained accurately by measuring the area under all the curves and then designating as an entity those components whose curves overlapped. To illustrate this, a sample of carbon-14 lactose, galactose, and glucose received a 24-hour chromatographic development. Although it was possible to locate and distinguish galactose and glucose qualitatively after scanning the strip (Figure 7), the curves overlapped and thus the total area contributed by galactose and glucose were taken as one and designated as the area for the total monoses. Table II lists the results and expected error for lactose and monoses.

Table II. Calculation of Specific Activity Using Summation Equation

	Area[a]			Calcd. Specific Activity (1×10^{-4} μc./μl.)	
	Lactose	Monoses	Total	Lactose	Monoses
Activity placed on paper (μc. $\times 10^{-4}$)	43.8	46.8	90.6		
Volume (μl.) placed on paper (2-μl. applications)	4	8	12		
No. 1 scan	71	76	147	10.96	5.85
No. 2 scan	63	68	131	10.90	5.88
No. 3 scan	61	67	128	10.78	5.94
Av. calcd. specific activity				10.89	5.89
Theoretical specific activity				10.96	5.85
% dev. from theory				−0.6	+0.7

[a] Count rate setting: 2500 c.p.m., slit width: 2.0 mm., Lasico planimeter no. 123 setting: 126.7.

Table III. Calculation of Specific Activity of Galactose-1-Carbon-14 from Scanning Curve Peak Heights Using Known Standards[a]

	Galactose-1-C-14 Standard Activity Placed on Paper (1×10^{-4} μc.)				Unknown Vol., μl., 2-μl. Application				Calcd. Specific Activity (1×10^{-4} μc./μl.)
	2.4	7.1	11.8	23.6	8	10	16	30	
Peak heights (units)[b]	2.2	5.3	8.2	16.3	4.5		6.7	12.5	0.61
	2.8	4.9	7.9	18.0	5.4		7.7	16.3	0.56
	2.0	5.9	9.4	15.9		4.0	6.4	12.0	0.57
Av. calcd. specific activity									0.58
Theoretical specific activity									0.59
% dev. from theory									−1.7

[a] Guide strip technique.
[b] Each division of chart assigned an arbitrary value of one.

The choice of the count rate setting on the ratemeter was an arbitrary one. The specific activities reported in Tables I and II were achieved at a setting of 1000 and 2500 counts per minute, respectively. While very satisfactory results were obtained at a setting of 250 counts per minute, a setting of 1000 counts per minute or higher was desirable, because the resulting curve became less irregular as the count rate setting was increased. Such a smooth line curve permitted the area under the curve to be measured with greater accuracy, since the boundaries of the curve were better defined. The area bounded by the curve and the base line (Figure 7) represented the activity of the component(s) above background, as the background count was compensated for by making it coincide with the base line of the recording chart.

The magnitude of the areas under the curves also influenced the error of the determination. The amount of activity placed on the filter paper for chromatographic resolution was sufficient to produce curves whose areas were of such a magnitude that a mechanical error of 2 to 3 units resulting from an area measurement was considered insignificant. In cases where the activity was greater than the limits of the count rate setting, either a higher setting was required or a dilution of the solution prior to spotting was made. However, for a quantitative assay based on area measurements, it was necessary, at all times, to scan the entire strip at the same count rate setting.

The error of the assay was also lessened by taking the average of three or more determinations. This was easily accomplished by scanning a strip three or more times, calculating the specific activity of the sugars using the area measurements obtained for each strip, and then averaging the results.

DETERMINATION OF SPECIFIC ACTIVITY USING KNOWN LABELED STANDARDS

When chromatographic separations of two or more carbon-14–labeled sugars was possible, but yet, because of their position on the chromatogram they yielded overlapping scanning curves due to the geometry of their activity, it was possible to determine the specific activities of these substances on paper chromatograms by making reference to known standards.

Experimental. Four carbon-14–labeled sugar solutions of known specific activity and three dilutions or concentrations of the unknown were spotted on 31.5 × 55 cm. rectangular strips of Schleicher and Schuell No. 589 White Ribbon filter paper. The seven spots were introduced along a line 7.5 cm. from one end at 3.5-cm. intervals in 2-μl. applications using a Gilmont ultramicroburet. A section 3.0 × 55 cm. on each edge of the strip was reserved for the spotting of an unlabeled standard of the sugar or sugars being assayed in sufficient concentration to become visible

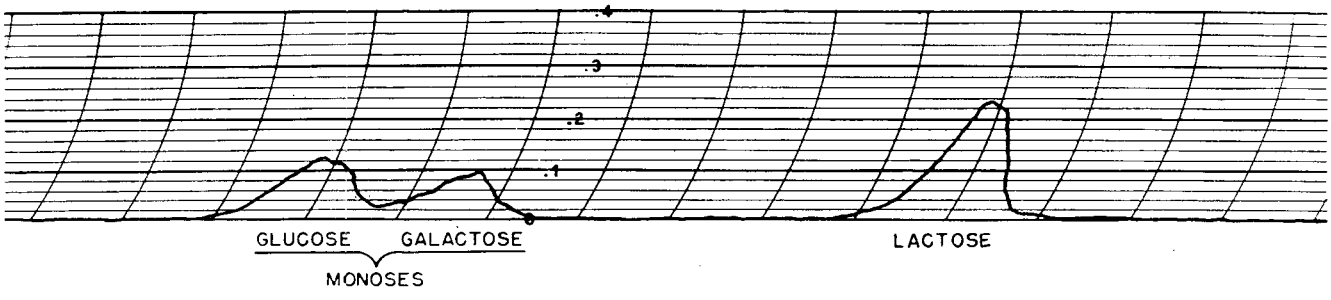

Figure 7. Scanning record of lactose-1-carbon-14, galactose-1-carbon-14, and glucose-carbon-14

Made after 24-hour chromatographic development in solvent system ethyl acetate (2.5)–pyridine (1.0)–water (3.5) Count rate setting, 1000 counts per minute
Slit width, 2.0 mm.

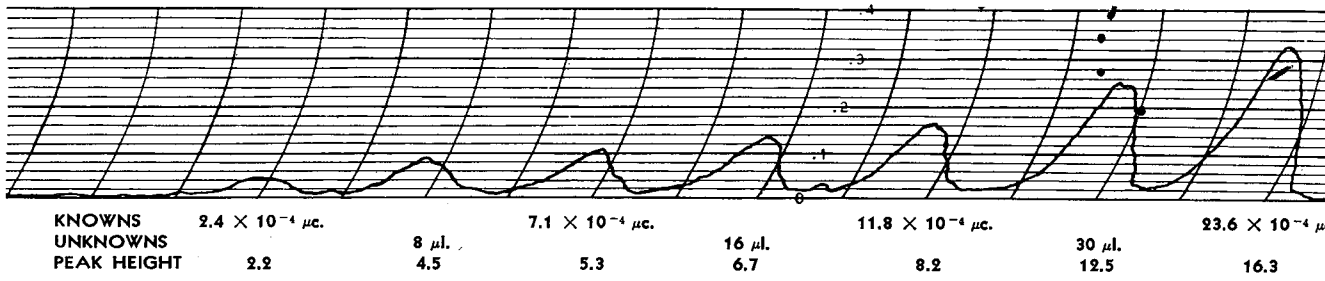

Figure 8. Typical scanning record using known standards in determining specific activity of carbon-14 labeled sugars

Source, galactose-1-carbon-14
Count rate setting, 1000 counts per minute
Slit width, 2.0 mm.

when reacted with a color reagent. This unlabeled compound served as a guide in order to locate the unreacted active components after solvent development. The chromatographic procedure was the same as that used in the method using a summation equation. The section of the chromatogram containing the four knowns and three unknowns was then cut out and scanned. A typical scanning record is shown in Figure 8.

The activity of the known labeled sugars used as standards varied depending on the source available. The lactose standards reported here were 8.95, 17.9, 35.8, and 53.7 × 10^{-4} μc., respectively; the galactose standards were 2.4, 7.1, 11.8, and 23.6 × 10^{-4} μc., respectively.

This procedure did not require the measurement of the area under the curves. The height of the curve showed a linear relationship to the activity recorded. The heights of the scanning curves of the standards were measured (the record chart contained 50 divisions and each division was assigned an arbitrary value of one) and a straight-line standard curve was prepared by plotting the activity of the known standards against the height of the scanning curves corresponding to these activities (Figure 9). The heights of the scanning curves of the unknowns were then measured and their specific activities calculated from the standard curve.

Results and Discussion. Table III lists the results and error using the second method in determining the specific activity of galactose-1-carbon-14 in a solution containing carbon-14–labeled lactose, galactose, and glucose.

When the concentration of the labeled sugar was sufficiently large, then as a substitute for the guide strip technique the chromatogram was treated (sprayed or dipped) with a color reagent which made the spots visible. Those portions of the chromatograms containing the labeled sugars were then cut out as a strip and scanned. However, the choice of color reagent was important. A reagent such as ammoniacal silver nitrate was excluded, because the deposition of free silver introduced an absorption factor which decreased the sensitivity of the spot recording considerably. Of necessity, then, was the use of a reagent which reacted with the labeled sugar to form a colored compound. Aniline hydrogen phthalate (*8*) proved to be adequate in this respect. The determination of the specific activity of lactose-1-carbon-14 using this reagent prior to scanning is listed in Table IV.

The standard curves obtained from known activities were not reproducible from one paper strip to another. Standards were applied each time on the same chromatogram with the unknowns. The necessity for doing this is illustrated by the data contained in Tables III and IV.

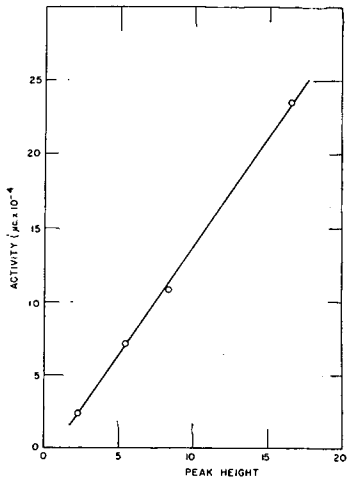

Figure 9. Typical curve obtained using known standards

Source, galactose-1-carbon-14
Count rate setting, 1000 counts per minute
Slit width, 2.0 mm.

These data show that for identical activities the peak heights varied for each scan. This may be in part attributed to the effect of residual solvent contributing to the absorption of the weak beta particles and in part attributed to the statistics of counting.

The activity of the standards used was governed by the specific activity of the carbon-14–labeled sugars available for this purpose and also by the activity of the solution under investigation. In the analyses reported in Tables III and IV a lower limit of 2.4 × 10^{-4} μc. was used for the galactose-1-carbon-14 standard and 8.95 × 10^{-4} μc. for the lactose-1-carbon standard. A val e of approximately 2.0 × 10^{-4} μc. of activity was sufficient to give an easily recognizable peak at a scanner count rate

Table IV. Calculation of Specific Activity of Lactose-1-Carbon-14 from Scanning Curve Peak Heights Using Known Standards[a]

	Lactose-1-C-14 Standard Activity Placed on Paper (1 × 10^{-4} μc.)				Unknown Vol., μl. 2-μl. Application			Calcd. Specific Activity[1] (1 × 10^{-4} μc./μl.)
	8.95	17.9	35.8	53.7	4	6	8	
Peak heights (units)[b]	7.5	17.2	30.5	49.0	14.1	22.5	31.0	4.2
	6.5	16.8	35.9	47.0	16.6	25.1	31.5	4.5
Av. calcd. specific activity								4.35
Theoretical specific activity								4.5
% dev. from theory								−3.3

[a] Color development of radiochromatogram with aniline hydrogen phthalate prior to scanning.
[b] Each division of chart assigned an arbitrary value of one.

setting of 1000 counts per minute and this value, as a rule, was used as the lower limit. The unknowns were spotted on the filter paper so that their peak heights fell within the same range as the unknowns. These conditions were established by preliminary scans.

As with the method using a summation equation, it was found that minimum errors were obtained when the average results of two or more scans were taken.

CONCLUSION

The application of either of the two methods to the determination of the specific activity of carbon-14–labeled lactose, galactose, and glucose results in an error no greater than 3%. However, of importance in these analyses is the chromatographic development to which the unknown solutions are exposed, prior to scanning. As with unlabeled sugars, the preparation of the sample, if from biological sources, is extremely critical. Interfering substances common to carbohydrate chromatography, such as salts and proteins which cause distortion of sugar chromatographic patterns, should be removed.

Relying on the extreme sensitivity of this apparatus—i.e., 2.4×10^{-4} μc. which is detected with ease—the techniques have been utilized to determine the purity of lactose-carbon-14 derived from a goat. These techniques now will be applied to a quantitative study of carbon-14 sugars in biological fluids in connection with lactose metabolism studies.

LITERATURE CITED

(1) Anderson, R. C., Brookhaven National Laboratory, Upton, N. Y., private communication.
(2) Demorest, H. L., and Baskin, R., ANAL. CHEM. **26**, 1531 (1954).
(3) Frierson, W. J., and Jones, J. W., *Ibid.*, **23**, 1447 (1951).
(4) Jeanes, A., Wise, C. S., and Dimler, R. J., *Ibid.*, **23**, 415 (1951).
(5) Jones, A. R., *Ibid.*, **24**, 1055 (1952).
(6) McFarren, E. F., Brand, K., and Rutkowski, H. R., *Ibid.*, **23**, 1146 (1951).
(7) Müller, R. H., and Wise, E. N., *Ibid.*, **23**, 207 (1951).
(8) Partridge, S. M., *Nature* **164**, 443 (1949).
(9) Rockland, L. B., Lieberman, J., and Dunn, M. S., ANAL. CHEM. **24**, 778 (1952).
(10) Soloway, S., Rennie, F. J., and Stetten, D., Jr. *Nucleonics* **10**, 52 (1952).
(11) Van Slyke, D. D., Steele, R., and Plazin, J., *J. Biol. Chem.* **192**, 781 (1951).
(12) Williams, R. R., and Smith, R. E., *Proc. Soc. Exptl. Biol. Med.* **77**, 169 (1951).
(13) Wingo, W. J., ANAL. CHEM. **26**, 1527 (1954).
(14) Winteringham, F. P. W., Harrison, A., and Bridges, R. G., *Analyst* **77**, 19 (1952).

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(Ethylenedinitrilo)tetraacetic Acid Chelation of Platinum Group Metals

Spectrophotometric Determination of Iridium

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The complex formed between iridium(IV) and (ethylenedinitrilo)tetraacetic acid may be used for the colorimetric determination of iridium over the range 5 to 60 p.p.m. An excess of (ethylenedinitrilo)tetraacetic acid is added to the sample of iridium(IV), the pH is adjusted to 11.4 to 12.6, the solution is warmed on a water bath for 10 minutes, cooled to room temperature, and finally the absorbance is measured at 313 mμ. The reaction between iridium(IV) and (ethylenedinitrilo)-tetraacetic acid is complete in 10 minutes at 80° to 90° C., and the color does not fade at room temperature in 12 hours. Other platinum group metals interfere and when large amounts are present, iridium must be separated before the determination. Nitrate also interferes but may be tolerated if it is duplicated in the standards.

THE spectrophotometric behavior of the palladous–(ethylenedinitrilo)tetraacetate complex has been discussed (*5*). In a continuation of the study of chelation of the platinum group metals with (ethylenedinitrilo)tetraacetic acid (ethylenediaminetetraacetic acid), it has been found that the absorbance maximum characteristics of basic solutions of (ethylenedinitrilo) tetraacetic acid and quadrivalent iridium may be used for the photometric determination of the latter.

Beamish and McBryde (*2*) have pointed out that only one satisfactory method for the colorimetric determination of iridium has been developed—namely, that of Ayres and Quick (*1*), who obtained a purple color through the interaction of iridium with a mixture of perchloric, nitric, and phosphoric acids. Beer's law is satisfied at 10 to 75 p.p.m. and other platinum group metals interfere only slightly. In their evaluation of this method Beamish and McBryde found a large average deviation. Sulfuric acid interferes and must be controlled in standards. A heating period of 1.5 hours is needed to develop the color, and the measurements are made in strongly acid solution.

Table I. Permissible Weight of Platinum Group Metals Giving Not More than 2% Error in Detection of 1.00 Mg. of Iridium

Metal	Milligram
Palladium(II)	0.1
Platinum(II)	0.7
Platinum(IV)	0.2
Rhodium(III)	0.07
Osmium(IV)	0.06
Ruthenium(III)	0.02

When (ethylenedinitrilo)tetraacetic acid is added to acidic chloride solutions of iridium(IV) and the solution is made strongly basic, it has been found in this laboratory that there is a decided shift of the absorbance toward the ultraviolet. A more detailed study of this complex is the subject of another paper (*6*).

In the development of the proposed method for the spectrophotometric determination of iridium there have been studied the pH of the sample, the adherence to Beer's law, the effect of time on the formation of the complex, the concentration of (ethylenedinitrilo)tetraacetic acid, and the effect of interfering ions of the platinum group metals.

Figure 1 shows the absorbance curves of solutions 0.000264*M* in iridium(IV) added as the chloride complex, and 0.00100*M* in (ethylenedinitrilo)tetraacetic acid added as the disodium salt.

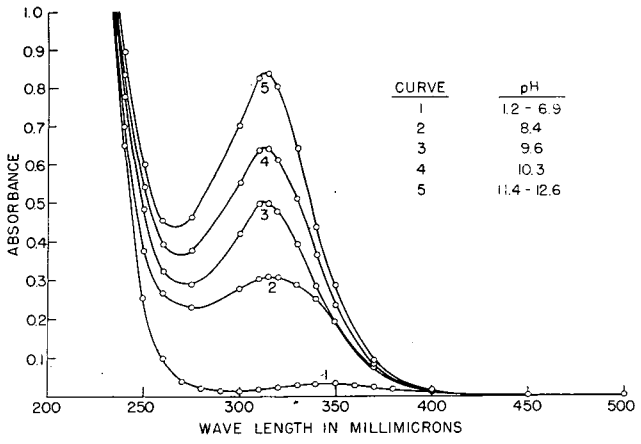

Figure 1. Absorbance curves for iridium–(ethylenedinitrilo)tetraacetic acid complex

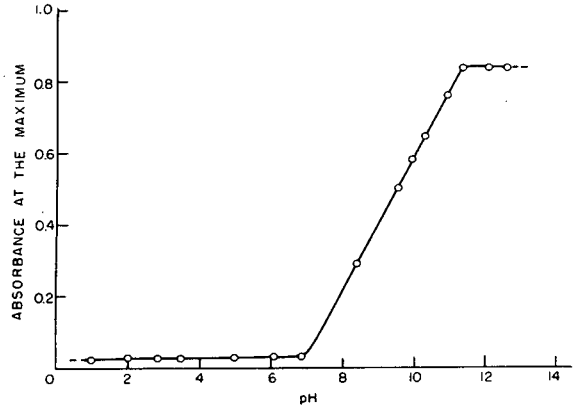

Figure 2. Absorbance maxima for iridium–(ethylenedinitrilo)tetraacetic acid complex as function of pH

The solutions were adjusted to the pH values shown with potassium hydroxide and the ionic strength was regulated at 0.1 with potassium chloride.

The reaction between iridium and (ethylenedinitrilo)tetraacetic acid proceeds slowly at room temperature and approximately 24 hours are needed for maximum absorption to develop. At 80° to 90° C. the reaction proceeds rapidly and is complete in less than 10 minutes. For example, the iridium concentrations found for a solution containing 65.2 p.p.m. of iridium heated at 85° to 90° C. were for t = 0, 37.8; t = 2′, 58.2; t = 4′, 63.7; t = 6′, 65.1; t = 8′, 65.3; t = 10′, 65.1; and t = 20′, 65.2 p.p.m.

Figure 2 shows that there are two pH regions in which the absorbance is nearly constant. The acidic range is not applicable for a spectrophotometric determination, because the absorbance is very low and there is no satisfactory peak in the visible or ultraviolet regions. In the pH range 11.4 to 12.6 a satisfactory maximum does occur which is constant over this range. In the pH range 8 to 10, absorbances shown in Figure 2 were difficult to measure accurately, as the solution became cloudy upon aging at room temperature or upon being heated at 80° to 90° C.

With absorbances measured at 313 mμ, adherence to Beer's law was tested for solutions in the pH range 11.5 to 12.6 containing 2.5 to 75 p.p.m. of iridium(IV). All solutions contained at least 2 or 3 millimoles of (ethylenedinitrilo)tetraacetic acid for each millimole of iridium, and solutions were heated for 10 minutes on a water bath to ensure equilibrium conditions prior to the measurements. Agreement with Beer's law is excellent except for a slight deviation below 3 p.p.m. The molar absorptivity for the iridium complex is equal to 3.2×10^3.

The effect of time upon the absorbance of a solution containing iridium(IV) and the complexing agent is shown in Figure 3. At room temperature a slow reaction occurred which did not reach equilibrium within 24 hours. The solution whose behavior is reported in Figure 3 was 0.000264M in iridium and 0.00100M in the disodium salt of (ethylenedinitrilo)tetraacetic acid. Its pH was 12.0. When a similar solution was heated at 80° to 90° C. equilibrium was reached in 10 minutes.

Also studied was the effect of the concentration of the (ethylenedinitrilo)tetraacetic acid on the rate of formation of the stable species having a maximum at 313 mμ. It was found that the rate of establishment of equilibrium conditions varied directly with the concentration of the complexing agent. However, because a 10-minute heating period at 80° to 90° C. produced equilibrium, it seemed unnecessary to provide more than 2 or 3 millimoles of (ethylenedinitrilo)tetraacetic acid for each millimole of iridium(IV) present. The complexing agent does not absorb appreciably at the wave length used for this determination. Therefore, an excess of this reagent does not interfere with the determination and need not be avoided.

The precision of the determination of iridium was determined at 10 and 75 p.p.m. of iridium. Ten determinations of the iridium in a solution containing 10.12 p.p.m. of iridium gave 10.02, 10.16, 10.18, 10.08, 10.21, 10.03, 10.09, 10.26, 10.04, and 10.19 p.p.m. Similarly 10 determinations of the iridium in a sample containing 75.6 p.p.m. of iridium gave 75.2, 75.1, 75.7, 75.8, 75.6, 75.9, 75.1, 75.8, 75.2, and 75.5 p.p.m.

The interference of other platinum group metals was studied by adding known amounts of hydrochloric acid solutions of osmium(IV), ruthenium(III), platinum(II), platinum(IV), rhodium(III), and palladium(II) to standard hydrochloric acid solutions of iridium(IV). Sufficient (ethylenedinitrilo)tetraacetic acid was added to provide 2 or 3 millimoles for each millimole of platinum group metal present. The pH was regulated to 11.4 to 12.6, the solution heated for 10 minutes, cooled to room temperature, and finally its absorbance measured at 313 mμ. The procedure followed with samples containing iridium and other platinum metals was identical with that for solutions containing only iridium. Table I shows the amount of other platinum group metals that cause a 2% variation in the absorbance in the spectrophotometric measurement of iridium. In the

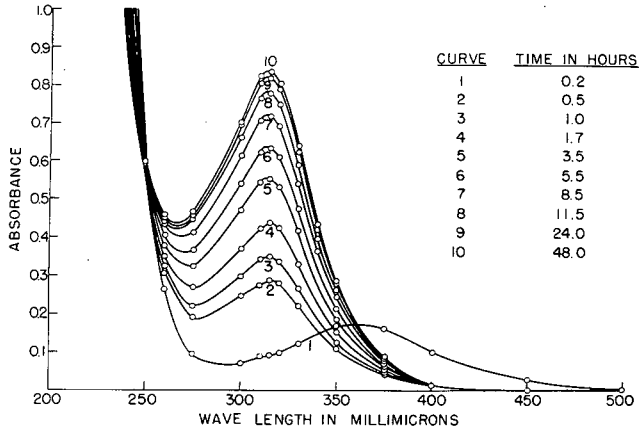

Figure 3. Variation in absorbance of iridium–(ethylenedinitrilo)tetraacetic acid complex upon aging

presence of larger amounts of other impurities, it may be necessary to separate the iridium prior to the final determination of the iridium photometrically. Sulfuric acid causes no noticeable change in the optical properties of these solutions. However, the presence of nitric acid interferes. There is an increase in absorbance in the region 260 to 330 mμ, which may be because of a reaction between chloride and nitrate ions, since this interference appears to be independent of the iridium concentration.

Recommended Procedure. If a significant quantity of trivalent iridium is present, warm the solution, containing the chloride, on the water bath for 1 hour, and pass chlorine gas slowly through it. Remove the excess chlorine by boiling the solution for 5 minutes. Add enough disodium salt of (ethylenedinitrilo)tetraacetic acid to provide 2 or 3 millimoles for each millimole of iridium, to the solution containing iridium(IV) in chloride solution. Adjust the pH to 11.4 to 12.6 with potassium hydroxide. Adjust the volume so that the concentration of iridium is 5 to 60 p.p.m. Heat for 10 minutes on a water bath at 80° to 90° C. Cool to room temperature and measure the absorbance in 1-cm. cells at 313 mμ.

EXPERIMENTAL

Apparatus. Absorbance measurements were made with a Beckman quartz spectrophotometer, Model DU, with 1.000-cm. matched silica cells. A constant sensitivity was maintained by use of variable slit widths.

pH measurements were made with a Beckman, Model H, battery-operated meter. A Beckman blue-tipped glass electrode was used in alkaline solutions.

Reagents. Iridium chloride was obtained from the American Platinum Works. Spectrographic investigation showed traces of rhodium present. Reagent grade perosmic acid, ruthenium chloride, platinous chloride, platinic chloride, rhodium chloride, and palladium chloride were used for the study of interferences. Spectrographic analyses indicated less than significant amounts of impurities. Solutions were analyzed and standardized by procedures from the Gilchrist-Wichers (*4*) scheme.

(Ethylenedinitrilo)tetraacetic acid was obtained as the disodium salt in analytical reagent grade from the F. W. Bersworth Co.

Preparation of Standard Solution. One-half gram of iridium chloride was dissolved in 500 ml. of 0.1*M* hydrochloric acid. The iridium was converted to the quadrivalent state by heating the solution on a water bath for 1 hour, while chlorine was bubbled into the solution. Excess chlorine was removed by boiling for 5 minutes. The total iridium in the standard solution was determined by a modified procedure of the Gilchrist-Wichers scheme (*4*). In a mildly alkaline aliquot the iridium was oxidized with sodium bromate, precipitated as the hydroxide, coagulated by heating, filtered, and the residue ignited first in air and then in a stream of hydrogen using a Rose crucible. The amount of iridium(IV) in the standard sample was determined by the iodometric procedure of Delépine (*3*). An excess of potassium iodide was added to an acidic aliquot of the standard solution, which reduced iridium(IV) to the trivalent state with the formation of an equivalent amount of iodine. The liberated iodine was titrated with a standard thiosulfate solution. Results of these two determinations indicated that the treatment with chlorine had resulted in the complete conversion of iridium to the quadrivalent state.

DISCUSSION

The use of (ethylenedinitrilo)tetraacetic acid in the photometric determination of iridium has several distinct advantages.

It is applicable over a concentration range (5 to 10 p.p.m.) in which other methods are less sensitive.

There is an almost linear relationship between concentration of iridium and absorbance over the range from 2.5 to 75 p.p.m.

The reaction is rapid and equilibrium is reached within 10 minutes at 80° to 90° C.

The color does not fade at room temperature in 12 hours.

Only one developing reagent is needed after the pH is regulated with potassium hydroxide.

An excess of (ethylenedinitrilo)tetraacetic acid causes no interference and need not be destroyed.

Sulfate ion is not detrimental.

This determination is performed in strongly basic solutions where no other method is applicable.

On the other hand, the chief limitation to the use of (ethylenedinitrilo)tetraacetic acid as a developing agent is the interference of the other platinum metals. The extent of interference is shown in Table I. In the presence of large quantities of interfering metals, it is necessary to separate the iridium first.

A second limitation to the method is the interference caused by the presence of nitrate ion. If more than a trace of nitrate is present in the sample it is necessary to add an amount of nitrate ion to each of the standards, equal to that in the unknown.

This method is primarily suggested for the rapid, accurate determination of chloride solutions of iridium(IV) containing only traces of other platinum metals.

LITERATURE CITED

(1) Ayres, G. H., and Quick, Q., ANAL. CHEM. **22**, 1403 (1950).
(2) Beamish, F. E., and McBryde, W. A. E., *Anal. Chim. Acta* **9**, 349 (1953).
(3) Delépine, M., *Ann. chim.* **7**, 277 (1917).
(4) Hillebrand, W. F., Lundell, G. E. F., Bright, H. A., and Hoffman, S. I., "Applied Inorganic Analysis," 2nd ed., pp. 339–83, Wiley, New York, 1953.
(5) MacNevin, W. M., and Kriege, O. H., ANAL. CHEM. **27**, 535 (1955).
(6) MacNevin, W. M., and Kriege, O. H., unpublished data.

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Emission Spectrometric Determination of Low Percentages of Zirconium in Hafnium

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The unique similarity in the chemical properties of zirconium and hafnium precludes the use of classical chemical methods for the determination of small amounts of zirconium in hafnium. In this paper, emission spectrometric procedures are described for the determination of zirconium in hafnium in the ranges 0.001 to 0.2% using conventional direct current carbon arc excitation and 0.01 to 0.5% using the conducting briquet excitation technique.

THE chemical properties of zirconium and hafnium are so strikingly similar that chemical analyses of mixtures of these elements can be made only by indirect methods, such as atomic weight (*4*, *7*, *14*) or density determinations (*11*). These procedures not only involve time-consuming operations and careful attention to experimental details, but they become increasingly inaccurate as the purity of the zirconium or hafnium increases. Moreover, pure binary mixtures are required in order to obtain reliable results.

These disadvantages do not affect optical emission and x-ray fluoresence spectrometric techniques and procedures covering the range from 0.003% hafnium in zirconium to 0.5% zirconium in hafnium have been described (*1*, *5*, *6*, *10*, *12*, *13*). None of the work published thus far has considered the determination of zirconium below 0.5% in purified hafnium. Emission spectrometric procedures are described here for the determination of zirconium in hafnium in the concentration range 0.001 to 0.5%.

SAMPLE FORM AND EXCITATION CONDITIONS

The mixed oxides were selected as the matrix for excitation, because they could be obtained readily from the metal and most of the chemical forms likely to be encountered. In addition, valid synthetic standards could be prepared readily by standard chemical operations.

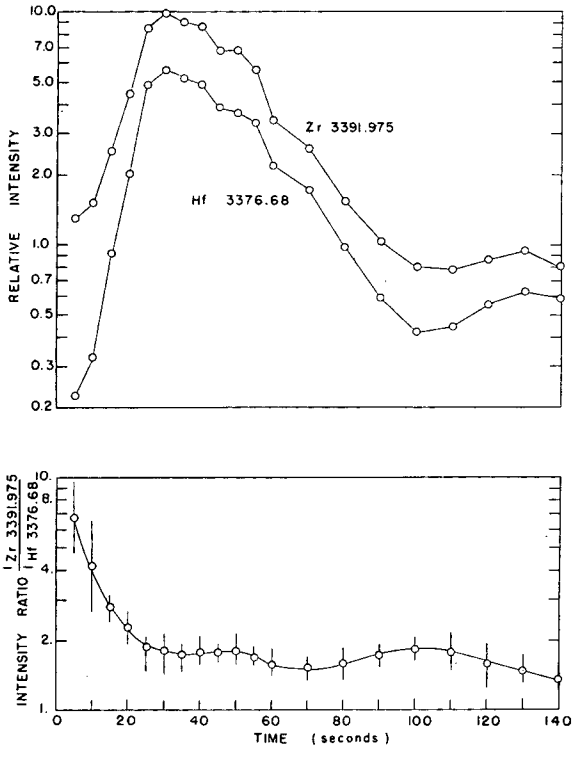

Figure 1. Moving plate studies with direct current arc excitation

Curves are average of quadruplicate runs

Excitation of the samples by the conventional direct current carbon arc and by the conducting briquet technique (*5*) was investigated. As expected, the direct current carbon arc excitation method could be extended to lower concentrations, while the conducting briquet technique gave more precise results.

The vaporization of the mixed oxides in a direct current carbon arc proceeds in a definitely erratic manner (*5*, *6*). Because there is a high degree of similarity in the volatilization behavior of zirconium and hafnium (Figure 1), acceptable internal standardization can be achieved by zirconium-hafnium line pairs which respond similarly to variations in excitation conditions. For the line pairs chosen, arc currents ranging from 7 to 18 amperes produced less than 10% deviation in the analytical intensity ratio.

The initial period of preferential volatilization of zirconium (Figure 1) enhances the sensitivity of detection at the expense of loss in precision. Figure 1 also shows that the major portion of the sample is consumed in about 60 seconds. Thus, termination of the exposure after 60 seconds produces maximum line to background ratios. The residual cap of carbides is not consumed after 180 seconds. For the conducting briquet excitation technique, the intensity ratios remain essentially constant during the excitation period (Figure 2); hence exposure times can be adjusted to obtain the desired exposure intensity.

PREPARATION OF STANDARDS AND EXPOSURE CONDITIONS

Synthetic standards were prepared from stock solutions of hafnyl and zirconyl sulfates. The solutions were prepared by dissolving the weighed oxides in hydrofluoric acid, fuming with sulfuric acid, and then diluting to standard volumes with distilled water. The solutions were mixed in the correct proportions and precipitated with ammonium hydroxide. After filtration, the precipitates were ignited overnight at 800° C. and then finely ground.

The pertinent experimental conditions and the equipment employed in the calibration experiments and analyses are summarized in Table I. Photography, microphotometry, and intensity ratio computations followed standard practices (*3*). The background readings used for correction purposes were obtained by averaging the readings on both sides of each line.

Table I. Operating Conditions for Determination of Low Percentages of Zirconium in Hafnium

Spectrograph	Jarrell-Ash Co. Wadsworth mounting spectrograph with original grating of 6.8 meter radius of curvature, 15,000 rulings per inch
Excitation sources	*A*. 60-cycle unidirectional overdamped condenser discharges; condensers charged to 940 volts and discharged through analytical gap once per cycle. Discharges obtained from Applied Research Laboratories Multisource, Model 2050, with following constants: Capacitance. 25 μfd. Inductance. 400 μh. Resistance. 65 ohms Initiator. High power Phase angle. 60° *B*. D.c. carbon arc, open circuit voltage 300 volts, arcing current 12 amperes, obtained from Applied Research Laboratories Multisource
Electrodes	
Lower	Overdamped d.c. discharges. Cylindrical briquet, 1/4 inch in diameter, formed as described in text D.c. carbon arc. Preformed graphite electrodes as shown in Figure 3, loaded with mixture of 5 mg. of oxide and 5 mg. of powdered graphite
Upper	Flat-end graphite rod, 1/8 inch in diameter, 1 inch long. Used with both excitation techniques
Analytical gap	4 mm.
Exposure time	80 seconds for overdamped d.c. discharges, 60 seconds for d.c. carbon arc
Slit width	30 microns
Emulsion	Eastman Kodak Co. Spectrum Analysis No. 1
Wave-length range	3000 to 3600 A., second order
Intensity modulation	8-step sector using 5 resulting darkest steps
Development	4 minutes at 20° C. in Eastman Kodak D-19 with continuous agitation
Microphotometry	Jarrell-Ash Console microphotometer, Model 2100, using 10-micron slit width
Emulsion calibration	Iron arc, two-step, preliminary curve method

With the zirconium 3391.975 A. line, the background could be read on only one side of the line.

The lower electrode employed for direct current carbon arc excitation (Figure 3) was purchased as a modified form of United Carbon Products Co., Inc., Model 101L preformed graphite electrode. The long constricted portion eliminated the striking of the arc to the edge of the supporting column. Use of this shallow electrode cavity caused the sample to begin volatilization very shortly after initiation of the discharge. With the unmodified electrode, volatilization was sometimes delayed for as much as 30 seconds after the arc was struck.

The cylindrical briquets employed with overdamped condenser discharge excitation were formed from 500 mg. of an intimate mixture of equal parts of the oxide sample and powdered flake graphite (National Carbon Co., Grade SP-1). These were pressed in an Applied Research Laboratories briquetting press with a load of 7000 pounds. Samples as small as 20 mg. can be excited by mixing the sample with an equal weight of powdered flake graphite and capping a graphite briquet with this mixture.

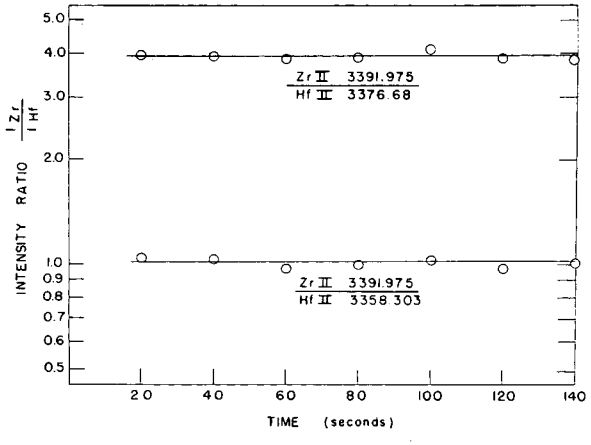

Figure 2. Moving plate studies with conducting briquet excitation technique

Curves are average of five exposures

SELECTION OF ANALYTICAL LINE PAIRS

Two analytical line pairs were selected for each excitation technique in order to limit the range of intensity ratios covered and thus improve the photometric precision. The wave lengths and excitation potentials of the lines and the concentration ranges covered by each pair are summarized in Table II. The analytical curves are shown in Figure 4. The points indicated on the curves represent the average values obtained from between three and seven exposures on individual plates. The data from the direct current carbon arc excitation are corrected for background, whereas those from the overdamped condenser discharge excitation are not.

The most sensitive zirconium line free of major interferences was found to be at 3391.975 A. The iron 3392.014 A. line is near enough to cause interference, but iron concentrations greater than 2 and 5%, for the direct current arc and conducting briquet techniques, respectively, were found necessary before any significant effect on the intensity ratios could be observed.

In the purest hafnium available, a line was detectable at 3391.975 A. when direct current arc excitation was used, but not when the conducting briquet excitation technique was employed. Residual correction values (*2*) obtained from conducting briquet exposures were significantly lower than values from direct current arc exposures, indicating the presence of an interfering line in the direct current arc spectrograms. The residual correction data obtained from both excitation methods were, however, rendered uncertain by the background contributed by the hafnium 3392.09 A. line [not listed in the MIT tables (*8*)]. To reduce this uncertainty, slit widths of 10 microns on the spec-

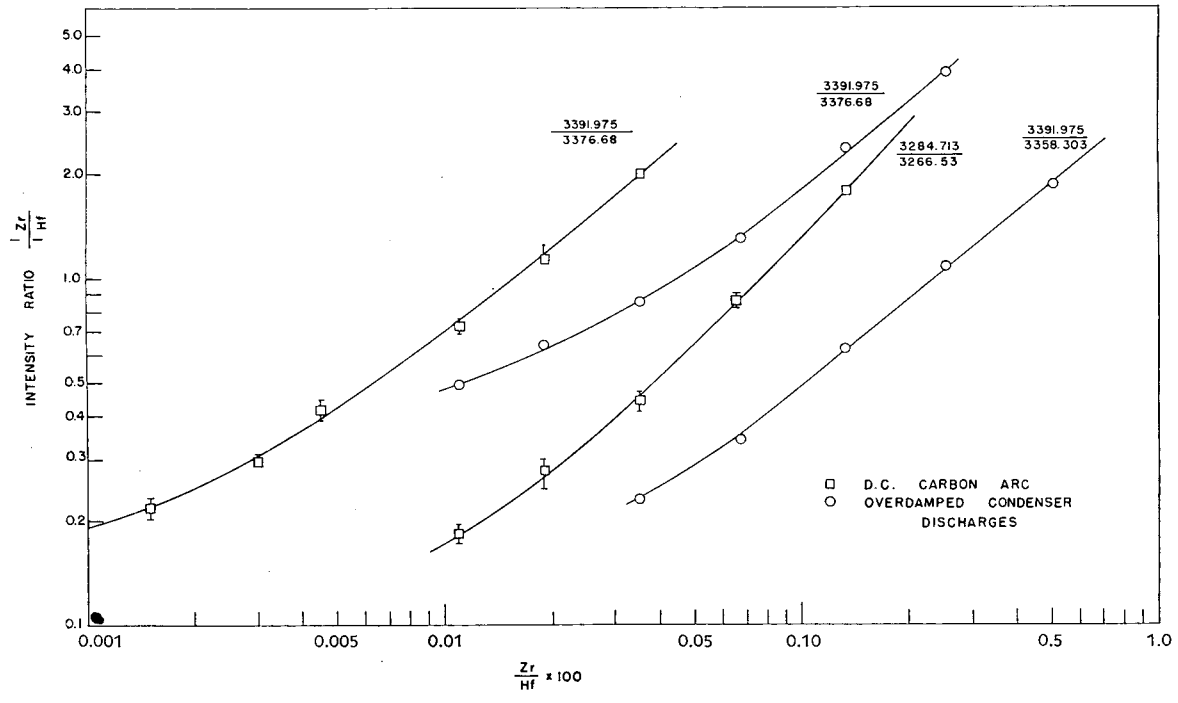

Figure 3. Analytical curves

Standards are corrected for residual zirconium content

trograph and 3 microns on the microphotometer were used. In order to provide corroborative residual correction data, two auxiliary, weaker zirconium lines at 2678.632 A. and 2700.131 A. were paired with the hafnium 2686.55 A. line and measured in direct current arc spectrograms. Although these zirconium lines were not detectable in the base material, this did not preclude their use for residual correction purposes (*9*). Accurate background corrections could be made for these lines. The residual corrections obtained from these lines in direct current arc spectrograms and the zirconium 3391.975 A. line in conducting briquet exposures all agreed within experimental error on two sets of standards prepared from different base materials (0.0029 ± 0.0003% and 0.0015 ± 0.0001%). The two series of standards gave identical analytical curves after correction for residual. The higher residual value obtained from the zirconium 3391.975 A. line in direct current arc spectrograms indicated the presence of an interfering line or an inability to make a rigorous background correction. Neither of these factors prevented the use of the 3391.975 A. line for analytical purposes, because accurate residual corrections were obtained from the independent measurements.

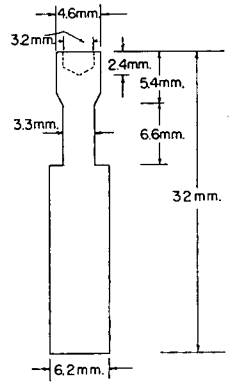

Figure 4. Graphite sample electrode for direct current carbon arc excitation

The analytical curve utilizing the zirconium 3284.713 A. line also shows some curvature at the lower concentrations, suggesting that the line is subject to interference. Supporting this is the fact that a value for the residual zirconium content obtained from this line is several times greater than the residual value found with the two auxiliary zirconium lines. This line was retained for analytical use despite this interference, because excellent precision was readily attainable and because it was conveniently located with respect to the other analysis lines.

SENSITIVITY, ACCURACY, AND PRECISION

The limit of quantitative detection was estimated using the concept that the minimum concentration measurable is reached when the line plus background emission is 1.3 times the background emission. With background corrections applied to the data from both the direct current arc and the overdamped condenser discharge, the estimated detection limits were 0.0003 and 0.003%, respectively. Obviously the nonlinearity of the analytical curves limits the precision attainable at these levels, so that the actual useful limits appear to be approximately twice these concentrations.

The comparison of analytical results as a means of establishing the accuracy of these methods was precluded by the lack of other analytical methods for determining zirconium in hafnium at this concentration. The accuracy was, however, demonstrated by the preparation of two different sets of standards at an interval of several months, which gave excellent agreement with respect to the shapes and positions of the analytical curves. The two sets of standards were prepared from different hafnium matrices, having as residual zirconium contents 0.0029 and 0.0015%, respectively. Analysis of the latter matrix with the direct current carbon arc procedure prior to its use in preparing the second set of standards yielded a zirconium concentration of 0.0014%.

Table II. Line Pairs Employed for Determination of Zirconium in Hafnium

Wave Length, A.	Excitation Potentials, Electron Volts	Concn. Range, % Zirconium
	D.C. Arc	
Zr II 3391.975 / Hf II 3376.68	10.72 / 11.01	0.001–0.04
Zr II 3284.713 / Hf II 3266.53	10.68 / 11.41	0.04–0.2
	Overdamped Condenser Discharge	
Zr II 3391.975 / Hf II 3376.68	10.72 / 11.01	0.01–0.15
Zr II 3391.975 / Hf II 3358.303	10.72 / 11.32	0.10–0.5

Precision data were obtained from single exposures of selected samples on individual plates which were exposed over a period of several weeks. Using overdamped condenser excitation, 20 exposures of a sample containing 0.045% zirconium yielded values of the coefficient of variation of ±3.0 and ±4.8 for the line pairs Zr 3391/Hf 3376 and Zr 3391/Hf 3358, respectively. With direct current carbon arc excitation, using eight exposures in each case, the line pairs Zr 3391/Hf 3376 and Zr 3284/Hf 3266, respectively, yielded coefficients of variation of ±8.0 with a sample containing 0.025% zirconium, and ±9.1 with a sample containing 0.051% zirconium.

LITERATURE CITED

(1) Birks, L. S., and Brooks, E. J., ANAL. CHEM. **22**, 1017 (1950).
(2) Cholak, J., and Story, R. V., *J. Opt. Soc. Amer.* **32**, 502 (1942).
(3) Churchill, J. R., IND. ENG. CHEM., ANAL. ED. **16**, 653 (1944).
(4) Claasen, A., *Z. anal. Chem.* **117**, 252 (1939).
(5) Fassel, V. A., and Anderson, C. H., *J. Opt. Soc. Amer.* **40**, 742 (1950).
(6) Feldman, C., ANAL. CHEM. **21**, 1211 (1949).
(7) Hahn, R. B., *Ibid.*, **23**, 1259 (1951).
(8) Harrison, G. R., "MIT Wavelength Tables," Wiley, New York, 1939.
(9) Hurwitz, J. K., ANAL. CHEM. **25**, 1028 (1953).
(10) Kingsbury, G. W. J., and Temple, R. B., *J. Applied Chem. (London)* **1**, 406 (1951).
(11) Larsen, E. M., Fernelius, W. C., and Quill, L. L., IND. ENG. CHEM., ANAL. ED. **15**, 512 (1943).
(12) Mortimore, D. M., and Noble, L. A., ANAL. CHEM. **25**, 296 (1953).
(13) Mortimore, D. M., and Romans, P. A., *J. Opt. Soc. Amer.* **42**, 673 (1952).
(14) Schumb, W. C., and Pittman, F. K., IND. ENG. CHEM., ANAL. ED. **14**, 512 (1942).

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Infrared Analysis of Bituminous Coals and Other Carbonaceous Materials

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The infrared spectra of many coal samples, principally bituminous coals, have been examined in studying their chemical composition, with several new structural assignments being made for the absorption bands in the spectra of high volatile bituminous coal. Interference due to minerals in the coal has been recognized. Kaolinite has been identified as the principal cause of a band hitherto assigned to aromatic ethers. Bituminous anthraxylons of the same rank from various parts of the world produce the same infrared spectrum. Spectra of bituminous anthraxylons, carbohydrate chars, coal hydrogenation asphaltene, petroleum asphalt, and gilsonite indicate quantitative, but not qualitative variations in chemical structures. Changes in the spectra of residues from vacuum distillation have been correlated with changes in chemical structure and with changes in rank. Visible region absorption appears to extend progressively farther into the infrared as the temperature is raised.

WORK on the infrared spectroscopic investigation of coal and coal products originated in Great Britain in recent years (*27*). Early investigators, Cannon and Sutherland (*6, 8*), were successful in obtaining spectra on coal sections and on mineral-oil mulls. They assigned some of the absorption bands to specific chemical bonds; Cannon (*4*) later made further assignments such as oxygen-containing groups, CH_2 and CH_3 groups, and both single-ring and condensed aromatic structures. Cannon also discussed the spectra of coals of different rank and the decrease in bands of oxygen-containing groups with increase in rank. Gordon, Adams, and Jenkins (*13*) have reported a technique for obtaining spectra of coal mulls, and Hadzi (*15*) has assigned chemical groups to absorption bands in spectra of tars and extracts from coal. Bergmann, Huck, Karweil, and Luther (*1*) successfully used the potassium bromide pellet technique for brown coals and higher rank coals, and on hydrogenated and oxidized coal. Work in this laboratory has been reported in part (*9, 11, 22*), and an article on the similarity between spectra of bituminous coal and carbohydrate chars (*10*) has been published. Also several articles have appeared recently on the infrared spectroscopy of coal and related substances (*2, 3, 14, 26, 29*).

The present report is a compilation of spectral studies on the chemical composition of coal, and includes experimental techniques, spectral band assignments, comparison of coal with products from coal and with other carbonaceous materials, and a spectral study of residues from the vacuum distillation of coal.

The infrared method can in situ detect the presence of chemical groups, preclude or limit the existence of proposed structures, and demonstrate similarities and differences in chemical structure of coals and coal-like materials. The spectra stand as characteristic properties that must be explained by any structure proposed for coal and coal-like substances. Infrared cannot detect differences in polymer sizes or crystallinity upon which many physical properties depend. It is complementary with the x-ray method that can be applied to study the physical structure of coals, but not their chemical composition.

EXPERIMENTAL PROCEDURE

Apparatus. Infrared spectra were obtained on a Perkin Elmer Model 21 spectrometer. The per cent transmittance values for the spectra of mulls were obtained with a fogged salt plate in the reference beam in order to obtain larger deflections at short wave lengths where the mulls scatter badly. The fogged plate, compared with a clear salt plate, transmits 20% at 3 microns, 27.6% at 5 microns, 39% at 7 microns, 46% at 9 microns, 54.8% at 11 microns, and 62.2% at 13 microns. Comparatively large slit widths were required to supply sufficient energy to the spectrometer (Slit Program 2: 0.078 mm. at 5 microns). Other samples that did not scatter badly were run in the conventional manner. Thin sections of coal were run *vs.* air, and asphalts, coal-tar, etc., were run with a polished rock-salt window as reference.

Samples. The preparation of thin sections of coal or coal constituents and the preparation of micronized powders for mulls, mulling agents, and cells have been described (*10*). Nujol has been used as the mulling agent for the work reported in this paper. The absorption bands labeled *N* in the figures signify that the bands are at least partly due to the Nujol. The potassium bromide pelleting technique has also been used; the spectrum of one pellet is shown in Figure 1. The principal objection to the potassium bromide technique is the production of OH absorption during the preparation of pellets, even if extreme precautions are used. This OH absorption cannot be accurately compensated by a blank. Where applicable the spectra obtained with thin

Table I. Ultimate Analyses of Coals and Other Substances

	Composition, Weight-Per Cent						
	C	H	N	S	O (diff.)	Ash	Moisture
Anthraxylon, Bruceton high volatile A bituminous, Pittsburgh seam	81.8	5.35	1.6	0.8	7.65	1.4	1.4
Coal, Bruceton high volatile A bituminous, Pittsburgh seam	75.5	5.2	1.5	1.5	7.0	7.2	2.1
Anthraxylon, Illawara high volatile A bituminous, New South Wales	82.2	5.32	1.86	0.55	7.24	1.58	1.25
Anthraxylon, Fulton low volatile bituminous, Huntingdon, Pa.	89.5	4.55	1.39	0.94	1.77	1.46	0.39
Anthraxylon, Hocloz seam low volatile bituminous, Liége, Belgium	88.2	4.51	1.52	0.90	3.95	0.27	0.65
Asphaltene from hydrogenation at 450° C. of Pittsburgh seam coal	89.5	6.4	1.5	0.1	2.5		
Gilsonite	85.4	10.11	3.16	0.37	0.59	0.15	0.22
Asphalt from Richfield Petroleum	86.74	7.37	2.82	1.66	1.41		
Char from sucrose at 250° C., Sn catalyst, 2500 lb./sq. inch gage hydrogen	72.1	4.6			23.3		
Cellulose	44.44	6.17			49.39		
Char from cellulose, 190° C.[a]	45.34	6.21			47.81	0.64[b]	
Char from cellulose, 250° C.[a]	71.54	4.79			22.48	1.19[b]	
Char from cellulose, 400° C.[a]	85.58	4.51			9.42	0.49[b]	

[a] Samples by courtesy of H. C. Howard (*24*).
[b] From corrosion of bomb.

sections or thin films (Figures 2 and 3) of coal or coal-like materials are preferred. The problem of radiation scattering by small particles is eliminated by the use of thin sections.

Specimens of coal-hydrogenation asphaltene, petroleum asphalt, and gilsonite were prepared by quickly melting the sample on a salt plate that had been heated. The sample was covered immediately with a salt plate to prevent any significant oxidation. Spacers between the two salt plates produced a film thickness of 0.038 mm.

The ultimate analyses of substances investigated are given in Table I.

DISCUSSION OF RESULTS

Spectral Assignments. A typical spectrum of a thin section of anthraxylon (vitrain) from high volatile A bituminous coal (Pittsburgh bed) is given in Figure 2. The principal absorption bands with tentative assignments are listed in Table II. Spectra were determined through the potassium bromide region to 25 microns, but no bands were found beyond 13.29 microns.

The last three long wave-length bands represent the types of aromatic substitution. The aromatic nuclei may be exclusively benzenoid; this assignment is complicated by the existence of strong bands in this region of the spectra of polynuclear aromatics. But it is not necessary to presume the presence of polynuclear condensed aromatic nuclei in order to explain the infrared spectrum. The assumed benzene rings may exist in hydroaromatic systems, but probably not in condensed polynuclear systems as in naphthalene, anthracene, etc. There are several reasons for

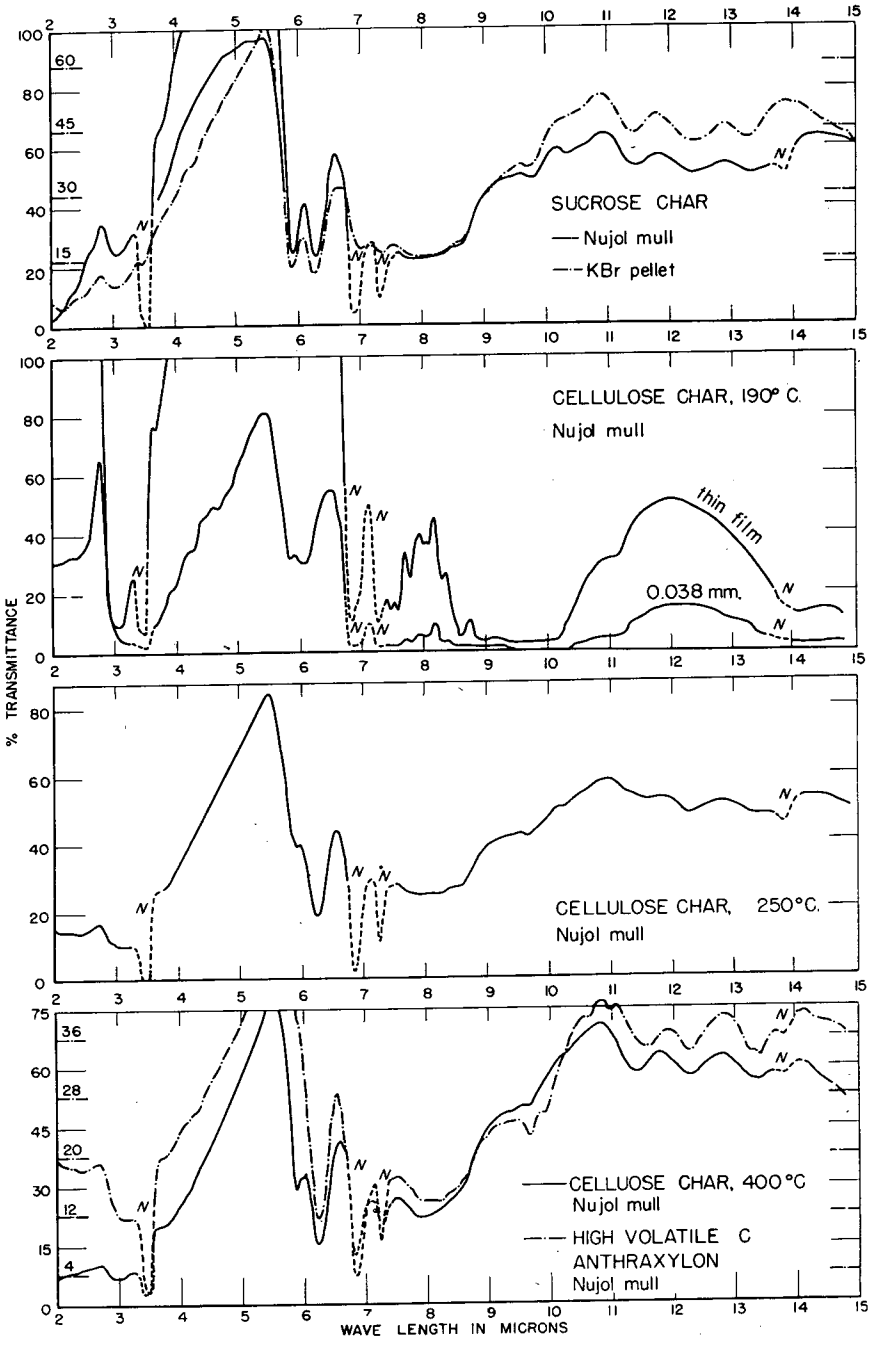


Figure 1. Infrared spectra of carbohydrate chars

assigning these bands exclusively to single, uncondensed benzene rings.

The three long wave-length bands do not exist exclusively in any one or any combination of spectra of polynuclear compounds investigated to date (*7, 21, 28*).

In the spectra of coal-hydrogenation asphaltenes, these aromatic bands become stronger instead of weaker. This condition is probably because of the concentrating of benzene rings effected by elimination of nonaromatic structures. The bands would be expected to weaken if they were due to condensed polynuclear aromatic groups that become partially saturated upon hydrogenation.

Phenols containing a condensed saturated ring, indanols, have been isolated from coal-hydrogenation products (*30*), but no phenols with a naphthalene or larger condensed aromatic ring system have been found.

A band is present near 6.65 microns in most spectra of aromatic compounds, but is found neither in coal spectra nor, significantly, in spectra of several hydroaromatic compounds with benzene nuclei.

The benzene carboxylic acids, identified by Roy and Howard (*23*), from the oxidation of bituminous coals have about the same types of substitution on the benzene ring as those herein ascribed to the infrared spectrum of the original coal.

The acids with polycyclic nuclei, discussed by Roy and Howard (*23*), have carbon-to-hydrogen ratios which indicate that the nuclei contain saturated rings.

To summarize the assignments, the spectrum of Pittsburgh anthraxylon indicates the following.

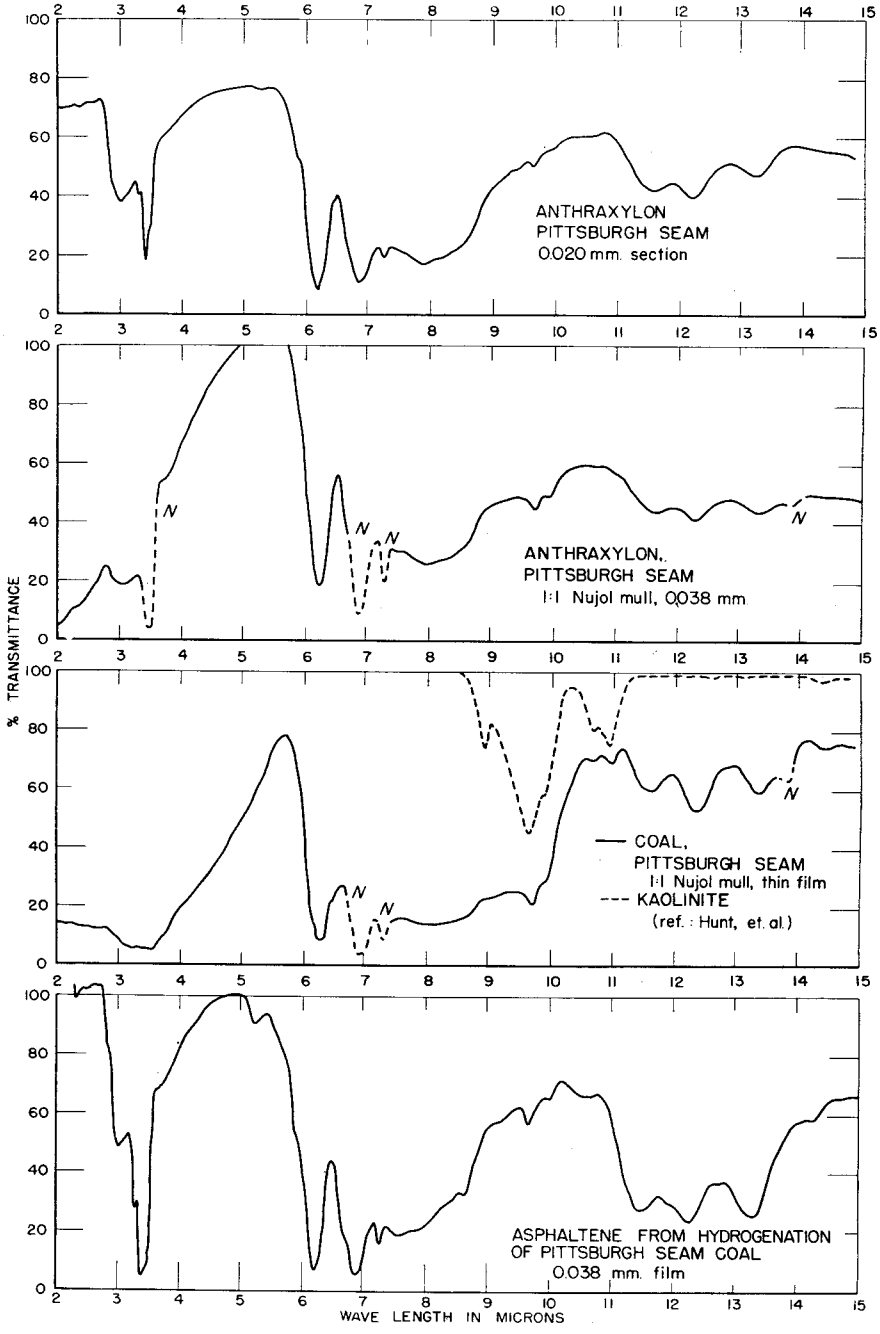
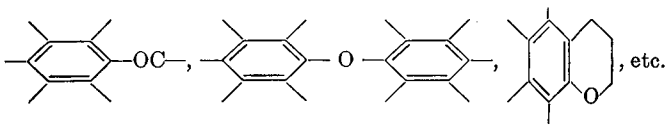


Figure 2. Infrared spectra of Pittsburgh-seam coal constituents and asphaltene from hydrogenation

High volatile coal contains large amounts of CH_2 groups in rings that may be naphthenic, hydroaromatic, and/or heterocyclic. CH_2 groups in long aliphatic chains are negligible. Ethyl groups are not excluded.

The aromatic nuclei may be exclusively benzenoid; possible benzene structures present are 1,2-di-(tetralin-type), 1,2,4-tri-, 1,2,3,4-tetra-(octahydrophenanthrene-type), 1,2,4,5-tetra-(octahydroanthracene-type), and 1,2,3,4,5-pentasubstituted. Some of the substituents are oxygen linkages on the benzene ring and may be phenols or



The other substituents on the rings are not predominantly CH_3 groups (weak 7.25-micron band) nor long alkyl chains (no 13.5 to 14.0-micron band); they may be hydroaromatic structures.

The per cent of aromatic CH is almost negligible compared with naphthenic CH. This condition does not mean that aromatic rings are scarce, but rather that these rings are highly substituted. However, the type of benzene ring indicated as being most plentiful is only trisubstituted (1,2,4-).

Meta linkages (1,3-disubstitution) are significantly absent, as are 1,3,5- and 1,2,3-substituted aromatics.

Monosubstituted benzenes are probably absent.

No unconjugated olefin bonds are indicated.

Aliphatic carbonyl groups are negligible.

The lack of intensity in the long wave length, aromatic bands indicates that partially substituted aromatic structures are not present in high concentration. These bands do not constitute a check on the concentration of completely substituted aromatics.

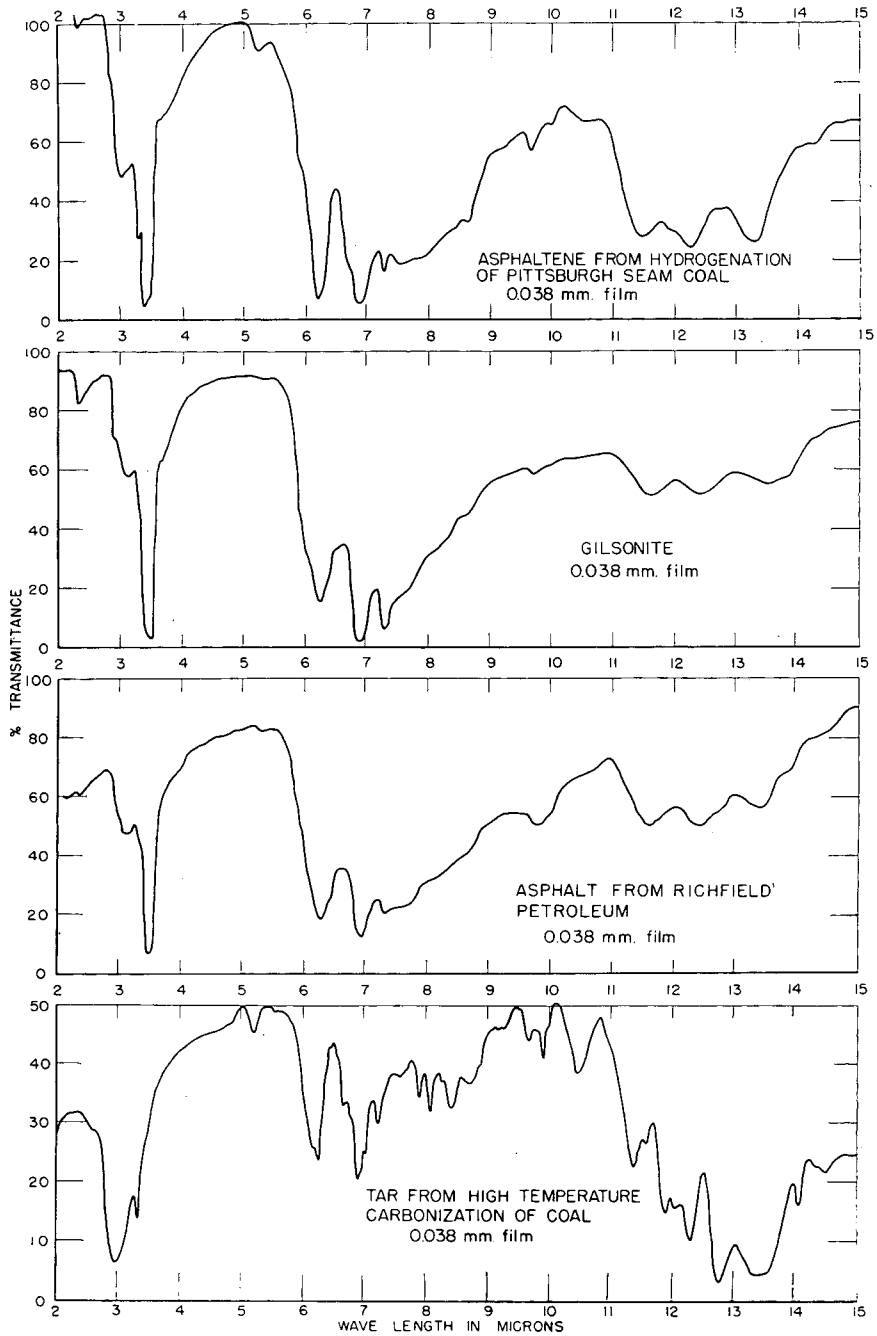


Figure 3. Comparison of infrared spectra of gilsonite and residues from coal and petroleum

All of the absorption bands in anthraxylon spectra, with the exception of part of the 9.67-micron mineral band, are attributable to combinations of carbon, hydrogen, and oxygen, as the same bands, with varying intensities, occur in the spectra of carbohydrate chars.

Specific assignments for the 3.0-micron OH band and the 6.19-micron band are discussed in the last section.

Mineral Absorption Bands. The spectrum of whole coal, Pittsburgh bed (Figure 2), differs markedly from the spectrum of the anthraxylon constituent. Instead of a sharp organic difference between anthraxylon and another constituent of coal, such as attritus or fusain, this spectral difference points to mineral content. The bands at 9.67, 10.0, 10.7, and 11.0 in Figure 2, which are in the spectrum of whole coal and not in that of anthraxylon, are assignable to minerals such as kaolinite (*9*, *11*, *18*). The assignment is supported by the great intensity of these bands in the spectrum (not shown) of a sample of semianthracite, which has high mineral content. The major differences between the spectrum of anthraxylon and the spectrum of attritus or fusain are therefore due to minerals. In addition to a qualitative difference in spectra, there is also a quantitative difference in the organic bands due to the dilution of the organic constituents by the minerals. Because of this mineral interference, infrared studies of the organic portion of the coal should be made on the low mineral anthraxylon constituent and not on the whole coal.

Table II. Spectral Assignments for Thin Section of Anthraxylon from High Volatile A Bituminous Coal

Microns	
3.00	Hydrogen-bonded OH or NH (see discussion in text)
3.30	Aromatic CH, weak
3.42, 3.49	Naphthenic and/or aliphatic CH bonds
5.22	Aromatic band, weak, prevalent in hydroaromatic compounds
5.87	C=O band, very weak shoulder
6.19	Very intense band; may be partly caused by a conjugated carbonyl structure such as in quinones (*5*). However, a quinone structure alone is probably not responsible (see discussion in text)
6.90	CH_2 groups. Very intense band, indicating large amounts of CH_2 groups in rings which may be naphthenic, hydroaromatic, or heterocyclic. Aliphatic CH_2 groups would produce a band at 6.83 microns, as in mineral oil (see Nujol spectrum in Figure 4). If present, this band is minor compared to the 6.9-micron band. The band is not resolvable under the higher resolution of a fluorite prism. In the spectra of Nujol mulls, the band becomes broader on the long wave length side, thus indicating the coal absorption at 6.9 in addition to the mineral-oil absorption band (Figure 4). Lack of a band at 13.5 to 14.0 microns indicates that aliphatic chains longer than $—CH_2CH_2$ are negligible. Mono- and polynuclear aromatic compounds with noncyclic substituents usually have strong bands between 6.2 and 6.9 microns that are not present in coal spectra nor in spectra of some hydroaromatic compounds.
7.25	CH_3 groups. This band is usually weak, indicating a small number of CH_3 groups.
8.0	Aromatic CO. Absorption in this region is typical of aromatic oxygenated compounds, such as phenols and aromatic ethers (phenoxy compounds). The disregarding of other possible CO-containing groups is based on the spectrum as a whole.
9.67	Aromatic band, intense in aromatic ethers. Also intense in minerals (see text).
11.64	1,2,4- or 1,2,4,5-substitution (or 1,2,3,4,5-) on benzene ring
12.25	1,2,4- or 1,2,3,4-substitution (perhaps 1,4-) on benzene ring
13.29	1,2-substitution on benzene ring

Not all of the 9.67-micron band is assigned to mineral content. Although weak, this band exists not only in the spectrum of anthraxylon, in which mineral content is very low, but also in the spectra of carbohydrate chars (*10*), in which no minerals exist. This weak band is usually found in spectra of aromatic compounds, particularly in the spectra of aromatic ethers. The weakness of this band in the spectrum of an anthraxylon thin section, which is particularly low in kaolinite (Figure 2), indicates a low concentration of aromatic ethers. Aliphatic ethers also are low as shown by weak absorption around 9.0 microns. No more than a minute fraction of the oxygen in Bruceton anthraxylon can be assigned to ethers.

Comparison of Different Coals. The anthraxylon components of a few coals of the same rank from various parts of the world have been investigated and found to have very similar spectra. A sample of high volatile A anthraxylon from the Illawara Field, New South Wales, gives the same spectrum as Pittsburgh high volatile A anthraxylon. Low volatile bituminous anthraxylons from Huntingdon, Pa., and from Liége, Belgium, give identical spectra.

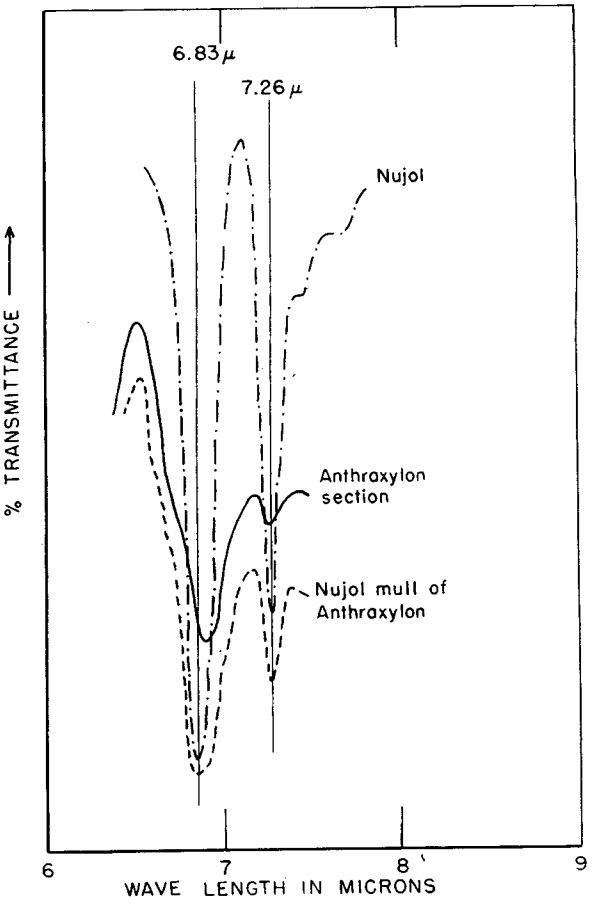
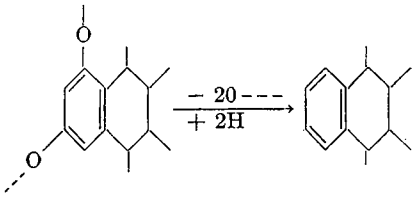


Figure 4. Infrared spectra of anthraxylon and Nujol in 7.0-micron region

Illustrates difference between aliphatic and nonaliphatic CH_2 groups

Differences among ranks of coal are apparent from an investigation of the spectra of anthraxylons from high volatile A, medium volatile, and low volatile coals (Figure 5). The OH band at 3.0 microns changes from a fairly definite band in the spectrum of high volatile A to an ill-defined broad band in the medium volatile to no band at all in the spectrum of low volatile anthraxylon. The same occurrence is noted for the broad carbon-oxygen band at 8 microns. The 6.19-micron band becomes sharper and possibly weaker in low volatile anthraxylon; this condition indicates that the band may be partly associated with oxygenated aromatic structures. As these oxygenated groups decrease in going from high to low volatile bituminous coal, the long wave-length aromatic bands at 11.64, 12.25, and 13.29

microns have increased in intensity and sharpness. This change indicates a concentrating of aromatic nuclei for low volatile coal and/or a decrease in the general background attributable to hydrogen-bonded structures which have been eliminated. The 11.64-micron band has shifted slightly and appears at 11.5 in low volatile coal. The 13.29-micron band has become the strongest and sharpest band of the three long wave-length bands in low volatile coal. This intensity change indicates that ortho-disubstitution has increased at the expense of higher-order substitution as oxygenated groups have decreased. These changes would be accomplished by some reaction such as



in going from high to low volatile coal.

On the side of the sharp 6.19-micron band in low volatile bituminous coal, a very weak band shoulder appears at 6.05 microns, which may be attributed to traces of carbonyl conjugated with an aromatic ring. This band may be present, but undiscernible, in the spectrum of high volatile bituminous coal, because of the broadness of the 6.19-micron band.

Oxygen content is known to decrease to about half between high and low volatile bituminous coal. Since absorption bands due to single-bonded oxygen are missing from low volatile coal, it appears that the oxygen must be present as double-bonded oxygen. In this case, the only assignable band is the 6.19-micron band, which may be due to a carbonyl group, perhaps in a quinone. No known quinone has its C=O band at a wave length as long as 6.19 microns (*12*), but strong hydrogen bonding of the C=O group may be responsible for this unusually long wave length.

The spectrum of low volatile bituminous coal is very similar to that of asphaltene from coal hydrogenation at 450° C. (Figure 3).

Asphaltene from Hydrogenation. The infrared spectrum (not shown) of asphaltene from coal hydrogenation at 400° C. is practically identical to the spectrum of anthraxylon, except for a decreased 3.0-micron OH band. The spectrum of asphaltene from coal hydrogenation at 450° C. (Figure 3) differs somewhat from that of anthraxylon and indicates the more intensive reaction expected at the higher temperature. The major changes in the 450° C. asphaltene spectrum are: a much weaker OH band (3.0 microns), better defined and stronger CH bands (3.5, 6.90, and 7.25 microns), weaker absorption due to fewer oxygenated aromatic bonds (8.0 microns), and stronger aromatic bands (11.64, 12.25, and 13.29 microns). In general, the only significant

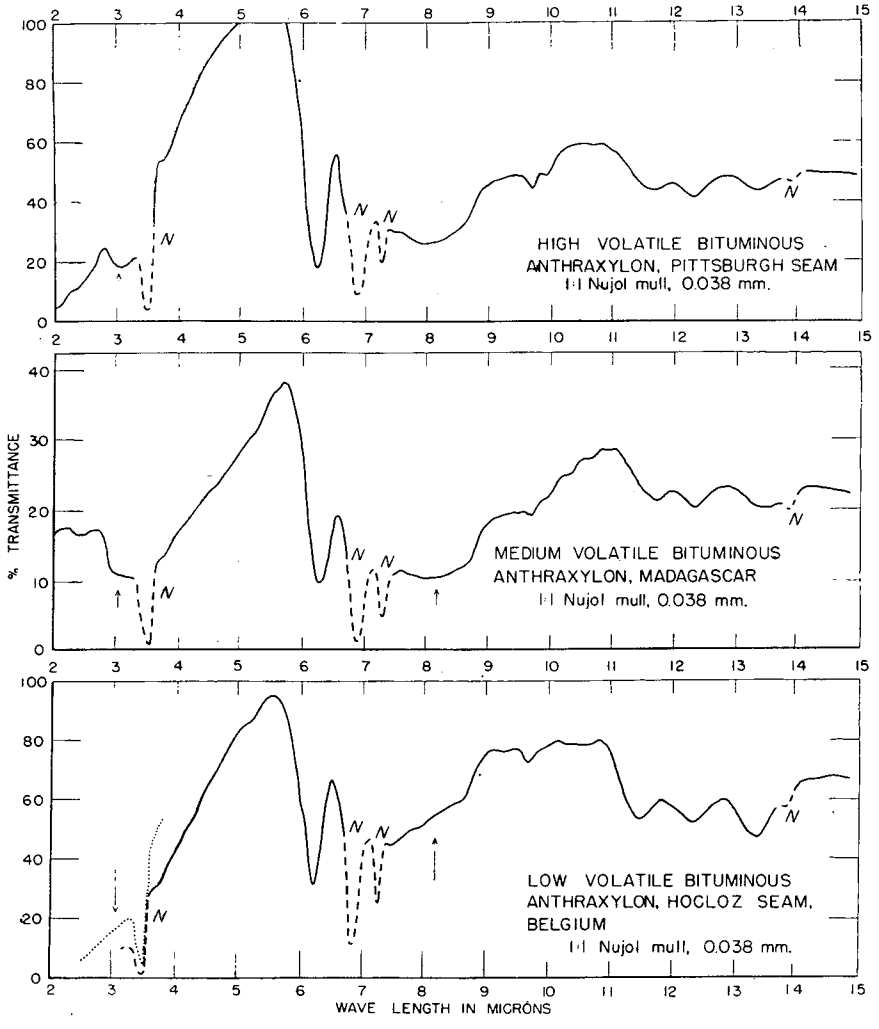


Figure 5. Effect of rank of anthraxylon on infrared spectra

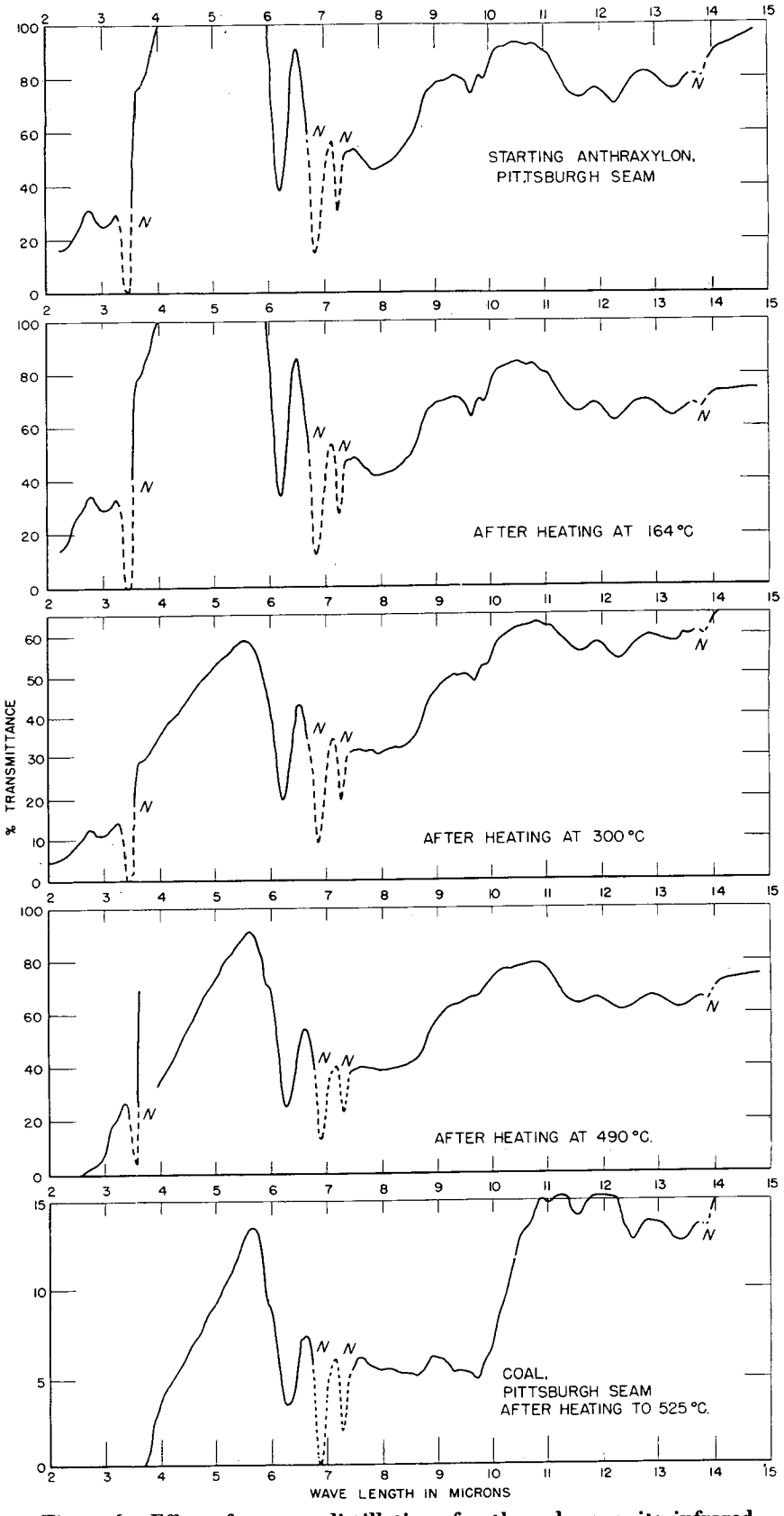


Figure 6. Effect of vacuum distillation of anthraxylon on its infrared spectrum

spectral changes are produced by elimination of oxygenated groups. Otherwise, the chemical configuration of anthraxylon appears to be altered very little in its liquefaction to asphaltenes by hydrogenation. Thus, support is given to the belief that hydrogen serves principally as a stabilizer in coal hydrogenation. The chief function of catalytic hydrogenation in the formation of asphaltene from coal is probably to saturate the free radicals or unsaturated compounds produced by the thermal decomposition of the coal (*25*).

Spectra of Richfield petroleum asphalt and gilsonite are presented in Figure 3 to illustrate their close comparison to coal asphaltene. It is apparent from these spectra that a definite chemical relationship exists between substances from coal and from petroleum. However, there are definite differences. Aromacity varies in the order: coal asphaltene > petroleum asphalt > gilsonite, as shown by the aromatic CH band at 3.30 microns and the other aromatic bands at 5.2, 9.67, 11.64, 12.25, and 13.29 microns. Paraffinicity varies in the opposite order, as shown by 7.25 and 13.9-micron absorption; lack of a 13.9-micron band in the coal asphaltene spectrum indicates negligible alkyl chains. Coal asphaltene displays more OH content than the others. In summary, the differences that exist indicate quantitative, but not qualitative variations in chemical structures.

A considerable chemical difference is indicated by the spectrum of tar from high temperature carbonization given in Figure 3 for comparison with asphalts and gilsonite.

Chars from Carbohydrates. The spectrum of anthraxylon from bituminous coal has been compared with spectra of sucrose and cellulose chars (*10*) (Figure 1). The char spectra have 5.90-micron C=O bands, and aliphatic CH bands weaker than for coal, as shown by the potassium bromide pellet spectrum of sucrose char. But the spectral similarities of coal are apparent and provide evidence that charring of sucrose and cellulose produces certain molecular structures similar to those in coal (*24*). The striking similarity in the hydrogenation reactions of bituminous coal and sucrose has been demonstrated by spectral and chemical comparisons of the product oils and asphalts (*22*).

The spectrum of sucrose char in Figure 1 was obtained at 250° C. under hydrogenation conditions (tin catalyst and hydrogen pressure of 2500 pounds per square inch gage). The char obtained from sucrose at 250° C. without hydrogenation conditions does not give a coal-like spectrum. Sucrose charred at 400° C. in nitrogen produces a coal-like spectrum. The last three spectra of Figure 1 are those of the cellulose chars prepared by Smith and Howard (*24*) at 190°, 250°, and 400° C. The spectrum of the brown, 190° C. char is not significantly different from the spectrum of cellulose (not shown) except that OH groups are slightly decreased. A radical change in structure obviously occurs between 190° and 250° C. Howard has noted the physical and chemical changes that accompany a strong exothermic reaction at approximately 250° C. The spectral changes produced by this reaction are striking. The aliphatic C—O band at 9.5 microns is very intense in cellulose and in the 190° C. char because of the large number of C—OH groups. In the 250° C. char this C—O band is practically eliminated. The OH stretching and bending frequencies at 3.0 and 6.0 microns respectively, are greatly decreased. The bands in coal at 6.19, 8.0, 11.6, 12.3, and 13.3 microns first appear in the 250° C. char; they become strong and well developed in the 400° C. char. At 400°C. the spectrum that is obtained is practically identical to a spectrum of high volatile C anthraxylon, reproduced on the same graph.

Vacuum Distillation of Coal. Proper assignments of infrared bands to specific chemical structures should be aided by a study of the vacuum distillation of anthraxylon. A considerable amount of chemical work has been done (*17*) with attempts to analyze the material given off by coal at various temperatures, but the residues have not been examined for possible changes in structure. By observing the behavior of the absorption bands of the residues at various temperatures, and thereby the thermal stability of the various chemical structures, assigning of absorption bands to specific structures appears possible. For example, disappearance of the OH band near 100° C. would indicate the band is due to water; disappearance around 500° C. may indicate minerals with OH groups; disappearance at other temperatures may indicate decomposition of specific organic groups.

Spectra were obtained at room temperature on Nujol mulls of portions of the Pittsburgh anthraxylon sample after it was heated at 164°, 200°, 250°, 300°, 370°, and 490° C. (Figure 6 gives four of these spectra.) Nothing significant occurs until 300° C., where the spectrum shows a decrease in the broad 8.0-micron phenoxy band. At 370° C. the spectrum shows a slight decrease in the OH band, and a definite decrease in the band at 8.0 microns. The 9.67-micron band is noticeably weaker.

At 490° C. more decomposition products were given off. The spectrum shows that the 9.67-micron band was destroyed and indicates that 8.0 to 9.0-micron absorption decreased further. The 3.0-micron OH band disappears. This disappearance is difficult to detect, because the carbonization process resulting from thermal degradation has broadened the absorption of the visible region until it spreads into the near infrared. Little change can be detected in the rest of the infrared spectrum; the intense 6.19-micron band is not changed perceptibly. The three long wave-length bands show a slight shift of the 11.64-micron band to shorter wave length and increasing intensity of the 13.29-micron band (ortho-disubstitution). Thus the spectral changes, noted between low and high temperature treatments, parallel the spectral differences between high volatile and low volatile bituminous anthraxylons.

A sample of residue from heating Pittsburgh whole coal under vacuum up to 525° C. was kindly supplied by the Coal Research Laboratory, Carnegie Institute of Technology. The spectrum of this sample (Figure 6) has the intense mineral band at 9.67 microns, which is characteristic of Pittsburgh coal. The aromatic bands with long wave length show definite changes, the trend of which change was noted for anthraxylon at 490° C. In particular, the band at 11.5 microns has shifted and the 13.29-micron band has become the strongest of the three. The absorption intensity of the entire spectrum increased tremendously.

Some conclusions can be drawn from this investigation. Because of the stability of the 3.0-micron OH band up to about 370° C., it is unlikely that any part of the band can be attributed to water, as such, in coal. The diminishing of the band may be attributed to the decomposition of phenols or of hydroperoxides to form water, which is always one of the products from the pyrolysis of coal. The decrease of the phenoxy band at 8.0 microns and of the aromatic ether–kaolinite band at 9.67 microns may be attributed to the elimination of phenols as such and/or the destruction of phenols and aromatic ethers or kaolinite. This investigation also indicates that very stable structures are responsible for most of the intense 6.19-micron band, as well as the 6.90-micron CH_2 band and the long wave-length bands at 11.64, 12.25, and 13.29 microns.

Another possible conclusion from these experiments is that the lack of appreciable change in the three aromatic bands with long wave length indicates that new polynuclear aromatic systems are not formed in raising the temperature to 490° C. Yet the intense absorption of the visible region appears to extend progressively farther into the infrared as the temperature is raised; proof of this extension has not been obtained because of the interference due to the scattering of the mulls. Kmetko (*19*) and McMichael, Kmetko, and Mrozowski (*20*) have observed recently the extension of absorption with rising temperature in photoconductive chars and have attributed it to an increase in π electrons. The existence of an electronic absorption band in bituminous coals is shown by the definite long wave-length edge

of the band located just below 2 microns. The sharp rise in transmittance values between the visible region and 2 microns is given in Table III.

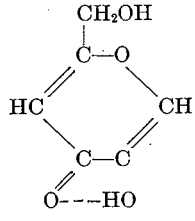
Table III. Infrared Spectrum of an Anthraxylon 0.020 Mm. Thin Section, from 0.85 to 2.65 Microns

(Indication of electronic absorption band)

Microns	% Transmittance
2.65	71.2
2.35	71.5
2.0	69.2
1.5	58.5
1.0	16.0
0.85	5.0

Since π-electron transitions are responsible for the opacity of most metals and of graphite, the implication in the heating of coal is that discrete polynuclear aromatic compounds are not formed upon heating, but that "graphitelike" (*4*) systems of unknown structure are formed. (Cannon has properly suggested that these systems are responsible for the properties of high rank coals and has observed the opacity of graphite in the infrared out to 15 microns.) Graphite is completely opaque to at least 25 microns, but all known condensed polynuclear aromatic hydrocarbons or oxygenated derivatives, no matter how complex, transmit in both the infrared and visible regions. It is unlikely therefore that the charring of opaque coal to a more opaque char would produce intermediate structures such as nonopaque polynuclear aromatic compounds.

Bands at 6.19 and 3.0 Microns. From the spectral behavior of the 6.19-micron band it is concluded that the band is produced by at least two different chemical bonds. In high volatile bituminous anthraxylon, the high intensity of this band can be attributed to conjugated carbonyl groups that are probably hydrogen-bonded and to phenoxy structures, principally phenols. In higher-rank coals, the slightly decreased intensity of the 6.19-micron band can be explained by the decrease in phenoxy groups; the band remains intense because of the carbonyl groups that are the major contribution to the band. The carbonyl groups are not necessarily hydrogen-bonded quinone carbonyls. It has been shown (*16*) that benzenoid compounds containing conjugated carbonyl groups with strong intramolecular hydrogen bonds—e.g., 2-hydroxy-4-methoxy acetophenone—can shift the carbonyl frequency to as far as 6.19 microns. Conjugation with an aromatic system is not necessary for the production of a 6.19-micron band by a strongly hydrogen-bonded carbonyl structure. For example, the authors have found that a γ-pyrone compound known as kojic acid, an intermediate from fungal degradation of glucose, produces its strongest band at 6.19 microns. From the structure,



it is expected that intramolecular hydrogen bonding would be very strong.

The band at 3.0 microns in coal is assigned to associated OH groups; NH groups are probably not present. The OH cannot be due to water, alcohols, or other saturated aliphatic structures containing hydroxyl groups, because other bands associated with these structures are not in the spectrum of coal. The spectral changes discussed under vacuum distillation also preclude these structures. Hydroperoxides may be present, but the majority of the associated OH groups at 3.0 microns are assigned to phenolic-type structures. A broad region of absorption extending to well beyond 3.0 microns indicates the presence also of OH groups that are very strongly hydrogen-bonded, and perhaps intramolecularly bonded with conjugated carbonyl groups. The spectrum of kojic acid has a very broad OH band centered at 3.3 microns that illustrates the effect of strong hydrogen bonding. It is felt that examination of the chemistry of this type of compound will be more fruitful in the elucidation of the structure of coal than will the study of polynuclear condensed aromatic compounds.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Bergmann, von G., Huck, G., Karweil, J., and Luther, H., *Brennstoff-Chem.* **35**, 175 (1954).
(2) Brown, *J. Chem. Soc.* **1955**, 744, 752.
(3) Brown, J. K., and Hirsch, P. B., *Nature* **175**, 229 (1955).
(4) Cannon, C. G., *Nature* **171**, 308 (1953).
(5) Cannon, C. G., private communication.
(6) Cannon, C. G., and Sutherland, G. B. B. M., *Nature* **156**, 240 (1945).
(7) Cannon, C. G., and Sutherland, G. B. B. M., *Spectrochim. Acta* **4**, 373 (1951).
(8) Cannon, C. G., and Sutherland, G. B. B. M., *Trans. Faraday Soc.* **41**, 279 (1945).
(9) Friedel, R. A., "Infrared Spectra of Coal and Other Carbonaceous Materials," Gordon Conferences on Coal, New Hampton, N. H., 1952 and 1954.
(10) Friedel, R. A., and Pelipetz, M. G., *J. Opt. Soc. Am.* **43**, 1051 (1953).
(11) Friedel, R. A., and Queiser, J. A., ANAL. CHEM. **26**, 434 (1954) (abstract).
(12) Fuson, N., and Josien, M., private communication.
(13) Gordon, R. R., Adams, W. N., and Jenkins, G. I., *Nature* **170**, 317 (1952).
(14) Gordon, R. R., Adams, W. N., Pitt, G. J., and Watson, G. H., *Nature* **174**, 1098 (1954).
(15) Hadzi, D., *Acad. Sci. et Ar. Sloven.* (Ljubljana) Class III, Ser. A, **3**, 99 (in English, pp. 108–11) (1951).
(16) Hergert, H. L., and Kurth, E. F., *J. Am. Chem. Soc.* **7**, 1622 (1953).
(17) Howard, H. C., "Chemistry of Coal Utilization," pp. 761–63, Wiley, New York, 1945.
(18) Hunt, J. M., Wisherd, M. P., and Bonham, L. C., ANAL. CHEM. **22**, 1478 (1950).
(19) Kmetko, E. A., *Phys. Rev.* **82**, 456 (1951).
(20) McMichael, B. D., Kmetko, E. A., and Mrozowski, S., *J. Opt. Soc. Am.* **44**, 26 (1954).
(21) Orr, S. F. D., and Thompson, H. W., *J. Chem. Soc.* **6**, 218 (1950).
(22) Pelipetz, M. G., and Friedel, R. A., "Hydrogenation of Bituminous Coal and Sugar at Elevated Temperatures," Division of Gas and Fuel Chemistry, 120th Meeting, ACS, New York, September, 1951.
(23) Roy, A. N., and Howard, H. C., *J. Am. Chem. Soc.* **74**, 3239 (1952).
(24) Smith, R. C., and Howard, H. C., *Ibid.*, **59**, 234 (1937).
(25) Storch, H. H., *Chem. Revs.* **29**, 483 (1941).
(26) Storch, H. H., *J. Inst. Fuel* **28** (April 1955).
(27) Sutherland, G. B. B. M., Fellgett, P. B., and Willis, H. A., *Proc. Conf. Ultrafine Structure of Coals and Cokes, Brit. Coal Utilisation Research Assoc.* **1944**, 330–3.
(28) Thompson, H. W., Vago, E. E., Corfield, M. C., and Orr, S. F. D., *J. Chem. Soc.* **6**, 214 (1950).
(29) Vucht, van, Rietveld, and Krevelen, van, *Fuel* **34**, 50 (1955).
(30) Woolfolk, E. O., Golumbic, C., Friedel, R. A., and Orchin, M., and Storch, H. H., Bur. of Mines Bull. **487**, 36 (1950).

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Characteristic Infrared Absorption Bands of Steroids with Reduced Ring *A*

3-Keto-5α- and 5β- Compounds

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A band for band analysis has been made of the infrared spectra of 27 3-keto reduced ring *A* steroids, 12 related structures, and 11 acetylated derivatives. The 5β-configuration gave rise to a combination of bands near 1337, 1299, 1103, and 1005 cm.$^{-1}$ Both allo and normal arrangements appeared to have characteristic absorption near 1254, 1154, and 965 cm.$^{-1}$ 3-Keto-5α- or 5β- and 3-hydroxy-5α- or 5β- steroids were differentiated in the 1100 to 1000-cm.$^{-1}$ region, as it has been demonstrated that ketones and hydroxyls at positions other than 3 have their effect usually at frequencies above 1050 cm.$^{-1}$ Contrariwise the identifying bands of 3-hydroxy-5α- or 5β- steroids were at frequencies below 1050 cm.$^{-1}$

IN A recent paper the infrared analysis of steroids containing a completely saturated ring *A* (3-hydroxy-5α- or 5β- compounds) were discussed (*4*). In that investigation it was mentioned that a useful approach to structural elucidation would be the application of a combination of characteristic frequencies which could permit assignment of a suspected steroid compound to a specific structural species. In the hopes of distinguishing 5α- and 5β- structures in the 3-keto series and differentiating 3-keto-5α- or 5β- and 3-hydroxy-5α- or 5β- compounds, consideration was given to all consistently occurring bands irrespective of intensity. There is no intention to assign specific interatomic vibrations to particular bands. The situation in the fingerprint region is complex, but a combination of characteristic bands would afford a high probability of assigning certain structural features to steroids with unknown structures. Thus, certain relationships of infrared absorption to chemical constitution in reduced ring *A* steroids with a hydroxyl or acetoxy group at position 3 were brought forth (*4*).

In the present study a second chemical species of reduced ring *A* steroids containing a ketone function at position 3 was examined. Certain infrared frequencies appeared to be characteristic for 3-keto-5α- and 5β- compounds and permitted ready differentiation from the 3-hydroxy-5α- and 5β- structures which also have spectral distinctiveness. The spectra of both groups of saturated ring *A* compounds were easily distinguished from those of steroids with conjugated ketones by the absence of absorption near 1666 cm.$^{-1}$ Therefore in the isolation of metabolites of α-, β-unsaturated steroids subjected to chemical or enzymic reduction, the intermediate (3-keto-5α- or 5β-) and terminal (3-hydroxy-5α- or 5β-) reduced compounds can be identified.

METHOD

The procedures and instrument employed were identical to those described in the preceding communication (*4*). Data from the pertinent spectra in Dobriner's atlas (*1*) have been incorporated with the findings from this laboratory. Both solution and solid film spectra were compared with the necessary precautions (*5*).

The infrared spectra of fourteen 3-keto, 5α-, twelve 3-keto, 5β-, five 3-keto-5α-acetate, and six 3-keto-5β-acetate steroids were analyzed. The influence of functional groups at other positions was elucidated from an examination of 13 spectra of less complex molecules.

ANALYSIS OF SPECTRA

Since the earliest frequency correlation studies on steroids, good evidence has existed for the interpretation that absorption bands in the 1100 to 1000-cm.$^{-1}$ region originate from C—O bonds (*2*). Although prominent bands in this region have been related primarily to hydroxyl groups at position 3, the C—O linkage of hydroxy and acetoxy functions at other positions could be expected to have an influence between 1100 and 1000 cm.$^{-1}$ No correlation of infrared absorption in this region and C—O bonds of hydroxyl groups at other positions of the steroid nucleus has as yet become apparent. If no significant interference of confusion can emerge from C—O linkages other than those of hydroxy groups at position 3, then perhaps alterations between 1100 and 1000 cm.$^{-1}$ could be useful for differentiating 3-hydroxy-5α- or 5β- from 3-keto-5α- or 5β- structures. Either a specific 3-keto effect or a spectral alteration arising from the absence of the 3-hydroxy group could suffice to differentiate 3-keto from 3-hydroxy reduced ring *A* structures in the 1100- to 1000-cm.$^{-1}$ region. With this in view 3-keto-5α- and 5β- and 3-hydroxy-5α- and 5β- (*4*) spectra were compared.

An examination of the spectra of ergostan-3-one, cholestan-3-one, androstan-3-one, etiocholan-3-one, and coprostan-3-one revealed no intense characteristic band between 1100 and 1000 cm.$^{-1}$ (Table I). Removal of the 3-ketone substituent—e.g., androstane, etiocholane, pregnane, etc.—did not result in a spectral alteration which could be consistently assigned to the loss of the 3-ketone group. In the normal configuration, etiocholane and pregnane, an intensification of bands near 1008, 1020, and 1064 cm.$^{-1}$ occurred which appeared to be characteristic of these molecules. In general, the 3-ketone function did not seem to exert a significant influence in the 1100- to 1000-cm.$^{-1}$ region.

A comparison of 3-ketone and 17-ketone spectra (androstan-17-one, etiocholan-17-one, and $\Delta^{2(\text{or }3)}$-androsten-17-one) indicated that the 17-carbonyl grouping does have an effect in the region under observation. Irrespective of the cis/trans relationship of rings *A* and *B*, all three 17-ketone steroids gave rise to intense bands near 1009 and 1052 cm.$^{-1}$ The corresponding 3-keto analogs were shown not to interfere with these bands. On the other hand the 17β-hydroxy analogs had a profound effect in the 1100- to 1000-cm.$^{-1}$ region.

The spectrum of etiocholan-17β-ol contained intense bands near 1031, 1039, 1052, and 1069, whereas the relative intensity of the 1008-cm.$^{-1}$ band appeared diminished. The spectrum of androstan-17β-ol had strong bands near 1028, 1049, and 1069 cm.$^{-1}$ Although substituents at position 17 in the carbon-19-steroids have a significant influence in the 1100- to 1000-cm.$^{-1}$ region, this would not necessarily eliminate use of this spectral zone for distinguishing carbon-21-3-keto and 3-hydroxy reduced ring *A* structures.

Absorption bands arising from steroids with two functional groups are also given in Table I. The two intense bands near 1010 and 1058 cm.$^{-1}$ in the spectrum of androstane-3,17-dione and at least one of the bands near 999 and 1018 in addition to that near 1050 cm.$^{-1}$ in the infrared curve of etiocholane-3,17-

dione could be assigned to an effect by the 17-ketone function. The monoketo-monohydroxy steroids gave a more complex picture. Androstan-17β-ol-3-one was responsible for bands near 1028, 1042, 1059, and 1078, whereas etiocholan-17β-ol-3-one had its major absorptions near 999, 1031, 1052, 1064, and 1072 cm.$^{-1}$ Differentiation of carbon-19-3-keto and 3-hydroxy reduced ring *A* compounds becomes difficult again.

As expected, the spectra of androstane-3,11,17-trione and etiocholane-3,11,17-trione contained strong bands near 1009 and 1050 cm.$^{-1}$ associated with 17-ketone vibrations. It has already been mentioned that the spectra of 3-ketone compounds have only weak bands at inconsistent frequencies in this range; this was also true for 11-ketone compounds.

A similar situation existed for the 20-ketone function. Weak bands at inconsistent frequencies occurred in the spectra of allopregnane, allopregnan-20-one, allopregnane-3,20-dione, and allopregnane-3,6,20-trione. In the latter compound little influence was related to the 6-ketone grouping. The strong band near 1008 cm.$^{-1}$ associated with the normal configuration of rings *A* and *B* appeared in the spectra of pregnane, pregnane-3,20-dione, pregnane-3,11,20-trione, and pregnan-17α-ol-3,20-dione (Table I).

In the more complex molecules with three, four, and five functional groups, assignments of absorption bands to certain hydroxyl groups were apparent (Table I). 21-Hydroxy steroids gave rise to a band between 1070 and 1079 cm.$^{-1}$ which did not depend on the type of side chain—e.g., 17α-hydroxy-α-ketol, α-ketol, or simply 21-hydroxy like allopregnan-21-ol-3,20-dione. That the 17α-hydroxy grouping has a distinct frequency is shown by the spectra of allopregnan-17α-ol-3,20-dione, 1088, allopregnan-17α-ol-3,11,20-trione, 1085, pregnan-17α-ol-3,11,20-trione, 1083, and pregnan-17α-ol-3,20-dione, either 1081 or 1090 cm.$^{-1}$ In 17α,21-dihydroxy steroids merging of 17α-hydroxy and 21-hydroxy vibrations occurred.

Two compounds were available with an 11β-hydroxyl substituent. Androstan-11β-ol-3,17-dione strongly absorbed near 1018, 1065, and 1088 cm.$^{-1}$ A shift to higher frequencies has occurred for the 17-ketone bands (1018 and 1065 cm.$^{-1}$) in this solid film spectrum. Allopregnane-11β,21-diol-3,20-dione gave rise to intense bands near 1076 and 1088 cm.$^{-1}$ The 1076-cm.$^{-1}$ band has been shown to originate in part from the 21-hydroxy vibrations. Therefore the 1088 cm.$^{-1}$ would appear to represent some activity of the 11β-hydroxyl function.

The findings may be summarized as follows: A 17-ketone has an effect near 1008 and 1052, whereas ketone groups at positions 3,6,11, and 20 had no comparable influence in the 1100 to 1000 cm.$^{-1}$ regions; a 17β-hydroxyl function was related to several bands near 1030, 1055, and 1075, whereas 11β-, 17α-, and 21-hydroxyl substituents appeared to be responsible for bands near 1088, 1085, and 1075 cm.$^{-1}$, respectively. A band near 1008 cm.$^{-1}$ was also related to the ring *A*/*B* stereoisomerism, the normal configuration giving an intensification of this band. Therefore in the compounds studied here hydroxyl groups at other positions than 3 exerted their more intense effects at frequencies above 1050 cm.$^{-1}$ In contradistinction 3-hydroxy-allo or normal structures absorbed below 1050 cm.$^{-1}$ (*4*). Some overlap may occur in solid film spectra for the 3β-, 5α- configuration and 17β-hydroxy or 17-ketone compounds. The latter could be distinguished by the characteristic pentacyclic ketone absorption, but the 17β- compound would be troublesome. Although 3α-, 5α-configurations absorbed between 1000 and 1009 cm.$^{-1}$ (*4*)

Table I. Influence of Ketone and Hydroxyl Functions of Dihydro Steroids in 1100- to 1000-Cm.$^{-1}$ Region

Compound[a]	Most Intense Frequencies between 1100 and 1000 Cm.$^{-1}$						
A. No Functional Groups							
Hydrocarbon							
Androstane	b	..			b		
Etiocholane	1009	1022			1065		
Ergostane	b	..			..		
Cholestane	b	b			..		
Pregnane	1006	1017			1063		
Allopregnane	b	..			..		
B. One Functional Group							
Monoketone							
Androstan-3-one	b	1022		..			
Etiocholan-3-one	1001	b		..			
Cholestan-3-one	..	..		b			
Coprostan-3-one	1006	..		b			
Ergostan-3-one[c]	b	..		..			
Androstan-17-one	1009	b		1055			
Etiocholan-17-one	1006	b		1051			
Δ^2-Androsten-17-one	1009	b		1049			
Allopregnan-20-one	b	..		b			
Monohydroxy							
Androstan-17β-ol	b	b	1028	1049	1069	..	
Etiocholan-17β-ol	1006	..	1031[d]	1052	1069	..	
C. Two Functional Groups							
Diketone							
Androstane-3,17-dione	1010	b		1058			
Etiocholane-3,17-dione	999	1018		1050			
Pregnane-3,20-dione	1005	b		b			
Allopregnane-3,20-dione	b	b		b			
Monoketone-monohydroxy							
Androstan-17β-ol-3-one	b	..	1028	1042	1059	1078	
Etiocholan-17β-ol-3-one	999	..	1031	1052	1064	1072	
D. Three Functional Groups							
Triketone							
Androstane-3,11,17-trione	1010	b		1049		b	
Etiocholane-3,11,17-trione	1007	1016		1049		b	
Allopregnane-3,6,20-trione	..	..		..		b	
Pregnane-3,11,20-trione	1002	1018		b		1072	
Monohydroxydiketone							
Pregnan-17α-ol-3,20-dione	1005				1062	..	1081[d]
Pregnan-11α-ol-3,20-dione[c]	1005				..	b	..
Allopregnan-21-ol-3,20-dione	b				..	1079	b
Allopregnan-17α-ol-3,20-dione	b				..	b	1088
Androstan-11β-ol-3,17-dione[c]	1015	..	1029		1065	..	1088
E. Four Functional Groups							
Monohydroxytriketone							
Pregnan-17α-ol-3,11,20-trione	1003					1071	1083
Allopregnan-17α-ol-3,11,20-trione[c]	b			1051[d]		..	1085
Dihydroxydiketone							
Allopregnane-11β,21-diol-3,20-dione[c]	b			b		1076	1088
Allopregnane-17α,21-diol-3,20-dione[c]	b			..		1073	b
Pregnane-17α,21-diol-3,20-dione[c]	1009			1059		1078	b
F. Five Functional Groups							
Dihydroxytriketone							
Allopregnane-17α,21-diol-3,11,20-trione[c]	b			1055		1076	..
Pregnane-17α,21-diol-3,11,20-trione[c]	1007			1041		1070	

[a] Most of spectra were studied in solution and can be found in Dobriner's atlas (*1*).
[b] Weak bands are present near these frequencies.
[c] Spectra obtained on solid films.
[d] Another band is present at a slightly higher frequency.

and in the present study a 17-ketone group also absorbed near this range, the pentacyclic ketone vibrations could easily differentiate the structures. It would appear that the 1100- to 1000-cm.$^{-1}$ spectra range can be useful for distinguishing carbon-21-3 hydroxy-5α- or 5β- and 3-keto-5α- or 5β- steroids.

In addition to this characteristic absorption between 1100 and 1000 cm.$^{-1}$ the 3-keto-5α- or 5β- steroids were differentiated from 3-hydroxy-5α- or 5β- compounds by the consistently occurring bands of weak to medium weak intensity near 1254 and 1154 cm.$^{-1}$ in both solid and solution spectra (Table II). Furthermore other bands, which aided in distinguishing 5α- and 5β- configurations in the 3-keto series, were also useful in separating 3-keto and 3-hydroxy reduced ring *A* structures. A combination of characteristic absorption bands related to the steric conformation of rings *A* and *B* occurred near 1333 (solid), 1340 (solution); 1302 (solid), 1297 (solution); and 1101 (solid), 1105 cm.$^{-1}$ (solution) in the spectra of the 5β- configuration (Table II). Two steroids out of 22, etiocholane and pregnan-17α-ol-3,11,20-trione, did not contain all three of this characteristic series of bands for the 5β- structure. Although many 5α- compounds had bands near these frequencies only the spectra of ergostane, Δ^2-androsten-17-one and allopregnan-20-one (three out of 28 steroids) contained all three bands.

It can be seen in Table II that another band appeared to occur with consistency in the spectra of 3-keto 5α- and 5β- steroids. Although the intensity of this band varied from medium weak to strong, it was observed near 963 (solution) and 967 cm.$^{-1}$ (solid) in the spectra of all allo compounds. The situation was somewhat variable in the normal structures; 11 of 12 solution spectra containing a band near 965 while the band was shifted out of this range in solid film curves.

In agreement with the observation on free 3-hydroxy-5α- or 5β-21-desoxy steroids containing no 17-hydroxyl group (*4*) was the finding that an intensification of the band near 1157 cm.$^{-1}$ occurred in the spectra of allopregnane-3,20-dione, pregnan-11α-ol-3,20-dione, and pregnane-3,11,20-trione.

A serious interpretation of the 1250-cm.$^{-1}$ region could not be undertaken for the acetylated structures because of the limitation of available compounds. However, some observations are worth noting. Most of the spectra of the acetates contained more than one band in the acetate region near 1258, 1225, and 1236 cm.$^{-1}$, the latter being of greatest intensity, whereas the other frequencies sometimes appeared only as side inflections. Readily distinguishable from this combination of frequencies was the singular absorption of allopregnane-11β,21-diol-3,20-dione-21-acetate, and the two 20α-hydroxysteroids near 1233 cm.$^{-1}$ The other 11β-hydroxysteroid, pregnane-11β,17α,21-triol-3,20-dione-21-acetate, gave rise to four bands, 1258, 1244, 1236, and 1230 cm.$^{-1}$ Furthermore the 21-ol-3,20-dione isomers seemed to absorb differently, the allo form being responsible for frequencies near 1253, 1238, and 1233, whereas the normal arrangement was near 1248 and 1230 cm.$^{-1}$ Finally the spectrum of the only available carbon-19-3-keto saturated acetate (etiocholan-17β-ol-3-one-17-acetate) had its major bands near 1255 and 1221 cm.$^{-1}$, different from all other curves.

After this work was completed, a paper appeared by Jones, Herling, and Katzenellenbogen (*3*) discussing the infrared spectra of ketosteroids below 1350 cm.$^{-1}$ The findings of this laboratory are in good agreement with theirs. The spectra of all 3-keto-5β-structures contained bands near 1337, 1295, 1104, and 1005 cm.$^{-1}$ Both 5β- and 5α-configurations gave rise to bands near 1254, 1154, and 965 cm.$^{-1}$ The 17-ketone grouping was responsible for strong absorption near 1008 and 1050 cm.$^{-1}$ The 20-ketone function did not have intense absorption in the 1100- to 1000-cm.$^{-1}$ region except for the expected band near 1005 cm.$^{-1}$ characteristic of 5β-structures.

Table II. Combinations of Characteristic Frequencies in Spectra of Reduced Ring A Steroids[a]

Compound[b]	Frequency Combinations Correlated with 3-Keto-5α- and 3-Keto-5β- Configurations							
Allo								
Androstane[c]	..	1302 S	..	1158 S	..	958 S		
Ergostane[c]	1334 M	1305 M	..	1159 M	1098 W	960 S		
Allopregnane[c]	1331 S	..	..	1159 M	1102 W	959 S		
Cholestane[c]	1338 S	1291 W	1251 M	1161 S	..	958 S		
Ergostan-3-one[c]	..	1306 W	1251 M	..	1101 W	958 M		
Cholestan-3-one[c]	1335 M	..	1253 M	1153 M	..	961 M		
Androstan-3-one[c]	1340 W	..	1255 M	1153 M	..	968 W		
Androstan-17-one[c]	1339 M	..	1259 S	..	1101 M	959 M		
Δ^2-Androsten-17-one[c]	1338 S	1295 M	1256 S	1151 W	1101 M	961 M		
Allopregnan-20-one[c]	1339 W	1292 M	1259 M	1155 S	1100 M	968 S		
Androstan-17β-ol[c]	1331 M	1305 W	1259 M	1159 M	..	958 M		
Androstane-3,17-dione[d]	1339 M	..	1258 W	1152 M	1101 W	969 W		
Allopregnane-3,20-dione[c]	..	1292 W	1250 M	1156 S	1101 W	964 W		
Androstan-17β-ol-3-one[d]	..	..	1258 M	1151 M	1101 W	960 W		
Androstane-3,11,17-trione[c]	..	1297 W	1255 M	1153 W	1107 W	967 W		
Allopregnane-3,6,20-trione[c]	0	0	0	0	1103 M	958 M		
Androstan-11β-ol-3,17-dione[d]	1334 W	1297 W	1249 W	1162 M	..	965 W		
Allopregnan-17α-ol-3,20-dione[c]	..	1300 W	1255 M	1152 M	..	968 W		
Allopregnan-21-ol-3,20-dione[c]	..	1297 M	1255 M	1152 M	1100 W	963 M		
Allopregnane-11β,21-diol-3,20-dione[d]	..	..	1252 W	1151 W	..	962 M		
Allopregnane-17α,21-diol-3,20-dione[d]	1340 M	..	1252 W	1152 M	1100 M	969 W		
Allopregnane-17α-ol-3,11,20-trione[d]	1333 M	..	1247 M	1161 W	1105 M	970 M		
Allopregnane-17α,21-diol-3,11,20-trione[d]	..	..	1250 M	1151 W	1101 M	971 M		
Allopregnan-20α-ol-3-one-20-acetate[d]	1335 W	1297 W	0	1152 M	..	966 W		
Allopregnan-21-ol-3,20-dione-21-acetate[d]	1339 W	1297 M	1253 M	1152 M	..	970 W		
Allopregnane-11β,21-diol-3,20-dione-21-acetate[c]	0	0	0	1162 M	..	971 W		
Allopregnane-17α,21-diol-3,20-dione-21-acetate[d]	..	..	1263 M	1152 W	..	969 W		
Allopregnane-17α,21-diol-3,11,20-trione-21-acetate[d]	1340 W	..	1253 M	..	1104 W	968 M		
Normal								
Etiocholane[c]	1342 S	1299 S	1253 M	1161 M	..	960 S		
Pregnane[c]	1336 M	1305 M	1250 M	1150 W	1108 M	962 M		
Etiocholan-17-one[c]	0	1292 M	1260 S	1159 M	1107 W	968 W		
Etiocholan-17β-ol[c]	1338 M	1308 M	1250 S	..	1109 M	958 M		
Etiocholan-3-one[c]	1340 M	1299 M	1256 M	1148 M	1103 M	959 M		
Coprostan-3-one[c]	1340 M	1297 M	1256 M	1151 M	1103 M	967 M		
Etiocholane-3,17-dione[d]	1337 M	1302 M	1250 M	1157 M	1101 M	..		
Pregnane-3,20-dione[d]	1335 M	1300 W	1250 W	1160 W	1100 M	..		
Etiocholan-17β-ol-3-one[c]	1339 M	1294 W	1253 M	1152 M	1104 M	970 M		
Etiocholane-3,11,17-trione[c]	1340 M	1295 M	1255 M	1148 M	1099 W	968 W		
Pregnane-3,11,20-trione[c]	1340 M	1299 M	1256 W	1152 S	1107 M	..		
Pregnan-11α-ol-3,20-dione[d]	1328 W	1307 W	1252 W	1161 S	1107 W	0		
Pregnan-17α-ol-3,20-dione[c]	0	0	0	0	1105 W	967 M		
Pregnan-17α-ol-3,11,20-trione[c]	0	0	0	0	..	968 M		
Pregnane-17α,21-diol-3,20-dione[d]	1335 W	1299 W	1253 M	1157 M	1101 M	..		
Pregnane-17α,21-diol-3,11,20-trione[d]	1332 W	1302 W	1261 M	1152 W	1099 M	..		
Etiocholan-17β-ol-3-one-17-acetate[d]	1339 M	1294 M	1255 S	1152 W	1109 M	0		
Pregnan-20α-ol-3-one-20-acetate[c]	0	1292 W	1256 W	1147 M	1105 M	0		
Pregnan-21-ol-3,20-dione-21-acetate[c]	1339 M	1292 M	1248 M	1149 M	1103 M	972 W		
Pregnane-17α,21-diol-3,20-dione-21-acetate[d]	1340 M	1299 M	1253 M	1159 W	1098 M	..		
Pregnane-17α,21-diol-3,11,20-trione-21-acetate[d]	1344 M	1299 M	1261 M	1152 M	1111 M	..		
Pregnane-11β,17α,21-triol-3,20-dione-21-acetate[d]	1337 M	1300 W	1258 M	1147 W	1105 W	..		

[a] Letters following frequencies denote relative intensity: S = medium strong to strong, M = medium weak to medium and W = weak; 0 = no determination available.
[b] Source of compounds not found in acknowledgment given in reference (*2*).
[c] Solution.
[d] Solid film.

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LITERATURE CITED

(1) Dobriner, K., Katzenellenbogen, E. R., and Jones, R. N., "Infrared Absorption Spectra of Steroids, An Atlas," Interscience, New York, 1953.
(2) Furchgott, R. F., Rosenkrantz, H., and Shorr, E., *J. Biol. Chem.* **163**, 375 (1946).
(3) Jones, R. N., Herling, F., and Katzenellenbogen, E., *J. Am. Chem. Soc.* **77**, 651 (1955).
(4) Rosenkrantz, H., and Skogstrom, P., *Ibid.*, **77**, 2237 (1955).
(5) Rosenkrantz, H., and Zablow, L., ANAL. CHEM. **25**, 1025 (1953).

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Flame Photometric Determination of Sodium, Potassium, Calcium, Magnesium, and Manganese in Glass and Raw Materials

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Use of the hydrogen flame attachment and photomultiplier tube with the Beckman Model B spectrophotometer in the analysis of soda-lime and borosilicate glasses, as well as various naturally occurring materials, such as sand, slag, and feldspar, is described. The range of concentration of the various components is wide and the accuracy obtained in the determinations varies, but in most cases is within 0.3 to 0.5% of the amount of the component present in the sample. A single disintegration of the material with no preliminary separations is employed in determining all of the components listed.

SOME flame photometric techniques involve preliminary separations from interfering elements, whereas other techniques call for comparison of the unknown solution with a standard which incorporates some or all of the interferences present in the unknown solution. Some authors add known or excess amounts of the interfering cations to both the unknown and the standard solutions, with or without first completely removing the interferent from the unknown solution by precipitation or by an exchange resin.

Although the flame photometric determination of the alkali, alkaline-earth, and certain other elements has considerably shortened analysis time, the preliminary separation and/or addition techniques detract from the speed and simplicity which might ultimately be attained. For this reason and because it was felt that the best accuracy could be obtained by making the standards as similar as possible to the sample solution as proposed by Gilbert and others (*6*), no preliminary treatment other than solution of the sample was employed in the method adopted and here described. Most of the flame photometric methods described in the literature employ a water or acid solution, or acid extraction of the sample for the determination of the alkali and alkaline-earth metals. Sintering is also used to effect solution of the sample for flame analysis. Where the sample is water- or acid-soluble, this technique is perfectly feasible. Acid extraction has the disadvantage of leaving part of the sample undissolved, and sintering of the sample is relatively time-consuming. The method of solution described herein takes less than an hour for most glasses and raw materials.

The powerful oxidizing action of boiling perchloric acid combined with the volatilizing action on silica and boron of hydrofluoric acid provides a sample solution technique which is sufficient for all glasses known to the author and for most glass raw materials.

The development of the photomultiplier tube for use in connection with the spectrophotometer has unquestionably extended the usefulness of flame analysis. Now it is possible to use the broad selection of sensitivities of the photodetector on the spectrophotometer in analyzing a single solution containing a number of components of widely varying emissivity. Although the Beckman Model B spectrophotometer with No. 9125 flame attachment was used in developing this method, the Beckman Model DU with flame attachment can be used, with no essential change in the method. The Model B is a direct-reading instrument and might be slightly faster in reading than the DU; the DU is undoubtably more sensitive and selective.

GENERAL CONSIDERATIONS

In all quantitative analyses, the qualitative make-up and approximate quantities of the components present in a given substance must be known, if the analysis is to have reasonable certainty of success. This information must also be known in making up a standard for a glass or other material which is to be analyzed by the method described. The needed information may be obtained from analyses of similar material, such as glass from the same tank, or examination of the flame spectrum of a totally unknown material will give a quick qualitative analysis for sodium, potassium, calcium, magnesium, and manganese, as well as lithium, strontium, copper, etc., and then a rough quantitative analysis may be run off immediately for the elements detected.

In the latter case, it is most convenient to make an approximately 0.1N hydrochloric acid solution of the material to be examined and compare it with solutions of each of the chlorides of sodium, potassium, calcium, magnesium, and manganese following in outline the procedure for glass analysis. In the case of a

Table I. Effect of Aluminum Salts on Intensity Readings of Synthetic Standard

Standard	Composition, Mg./L.		Al_2O_3, Mg./L. None	Al_2O_3, Mg./L. 50	Net Effect of Additive, %
			Intensity Readings		
S_1, S_2	CaO	300.0	64.5	60.0	− 7.0
	MgO	173.4	60.0	60.0	0.0
	Na_2O	700.0	60.0	60.0	0.0
	K_2O	10.0	62.0	62.0	0.0
			100 mg./l.		
E_1, E_3, L	CaO	400.0	66.0	60.0	− 9.1
	MgO	150.0	61.0	61.0	0.0
	Na_2O	700.0	60.0	60.0	0.0
	K_2O	10.0	63.0	63.0	0.0
			200 mg./l.		
Sa	CaO	500.0	71.0	61.0	−14.1
	MgO	17.2	60.0	60.0	0.0
	Na_2O	750.0	60.0	60.0	0.0
	K_2O	60.0	60.0	60.0	0.0

Table II. Effect of Iron Salts on Calcium and Magnesium Readings of Synthetic Standard

Standard	Composition, Mg./L.		Fe_2O_3, Mg./L. None	Fe_2O_3, Mg./L. 10	Net Effect of Additive, %
			Intensity Readings		
E_1, E_3, L	Al_2O_3	100.0	62.5	62.5	0.0
	CaO	400.0	62.5	62.5	0.0
	MgO	150.0			
	Na_2O	700.0			
	K_2O	10.0			
S_1, S_2	Al_2O_3	50.0			
	CaO	300.0	62.0	62.0	0.0
	MgO	173.4	62.0	62.0	0.0
	Na_2O	700.0			
	K_2O	10.0			

glass, a convenient concentration of sample is 0.5 gram per 100 ml. Using this concentration as the feasible upper limit, the solutions of a single salt used as a standard in the preliminary quantitative analysis need be no stronger. Starting with the highest possible percentage of a given component, which can be in the unknown, the standard salt solution is compared, then diluted down volumetrically until it reads within a factor of 2 of the unknown, and an approximate percentage of the desired component is calculated. In this manner, all of the elements which show up in the flame spectrum of the particular unknown in question are determined.

Even though they do not show up in the flame spectrum, the presence of any other elements in the sample under examination should be known, so that their inclusion in the standard may be decided upon on the basis of their effect on the emissivity of the sample. Thus in the case of the totally unknown material, an additional scheme of qualitative analysis probably has to be run through, to obtain the information necessary for making up a standard. Such a standard ideally should be similar in composition to the unknown solution with respect to the concentration of both the flame-emittent elements and those elements affecting the emissivity of the unknown.

Certain considerations simplify the making up of the standard. By definition, this excludes from consideration components that are present in too low a concentration to show up in the flame spectrum, are not themselves excited by the flame, have no enhancing or depressing effect, and do not contribute noticeably to the flame background. This is illustrated later in the make-up of a glass standard. Because in many cases the relationship between the element concentration and the emission of that element is linear over a fairly wide range of concentration, especially in the more dilute solutions, a further simplification is in the leeway that may be allowed between the sample and standard concentrations of emissive elements. This has been shown by numerous investigators, including Gilbert, Hawes, and Beckman (*6*), Close, Smith, and Watson (*4*), Close and Watson (*5*), and Beckman Instruments (*2*). Still a third simplification lies in the fact that the interference effect of any given component is but a small fraction of the magnitude of the concentration of the component, and therefore a small variation in the concentration of the interferent results in a negligible change in the interference effect.

The use of a blank in any given determination is an attempt to eliminate the background effect. Theoretically the background effect at any given emission peak should be very nearly the same for the sample being measured as for the synthetic standard. Thus the same amount may be subtracted from both the unknown and the standard readings in order to compensate for the energy reaching the photocell which is not relevant to the element in question.

EXPERIMENTAL WORK

Aluminum does not show up sufficiently in the flame spectrum at the concentration of 0.5 gram per 100 ml. of sample to be measured on the Beckman Model B, even in the case of feldspar and nepheline syenite, which contain some 20% alumina. However, Mosher, Bird, and Boyle (*7*) have shown that aluminum in solution has a pronounced depressive effect on the calcium emission when calcium is present alone in solution and a somewhat lesser depressive effect when magnesium and iron are also in the solution. The depressive effect of aluminum on calcium has also been observed by Brabson and Wilhide (*3*). Accordingly, the effect of aluminum additions to the synthetic standard was studied. Table I shows that addition of aluminum depresses the calcium emission, with no effect on the other components. This depression is proportional to the amount of aluminum present, but is also affected by the other components.

Figure 1 shows the effect of perchloric acid additions to the synthetic standard. The data for these curves were obtained by adding small increments of the 72% acid to the E_1, E_3, L synthetic standard. None of the components other than calcium and magnesium changed in intensity and the dilution effect of the additions was negligible. In the case of calcium, a limiting or saturation value is reached at 0.01*N* perchloric acid; magnesium reaches a saturation value at about 0.05*N*. Continuation of additions of perchloric acid results in no measurable further increase in emission intensity of either calcium or magnesium. The increase in calcium intensity is 12%, and the increase in magnesium intensity is 10% on the basis of the original emissions in the particular solution examined.

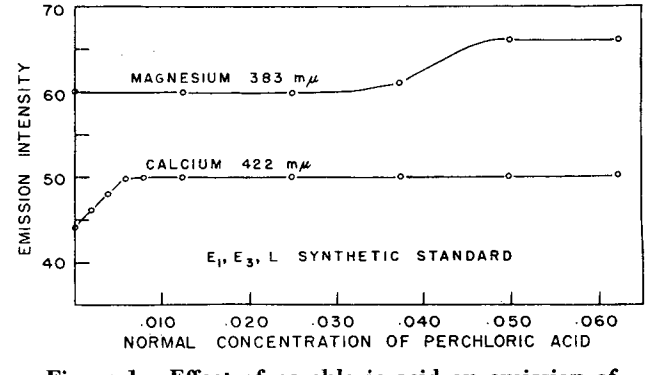

Figure 1. Effect of perchloric acid on emission of calcium and magnesium in synthetic standard

Mosher, Bird, and Boyle (*7*) have observed that iron depresses the calcium emission to some extent. Close, Smith, and Watson (*4*) have also shown that sulfate and manganese affect the calcium and magnesium emission in hydrogen flame spectrophotometry. Accordingly, the effect on the synthetic standard of iron and sulfate in the concentrations usually present in glass was studied with a view to their possible exclusion from the standard.

Table II shows the results of additions of iron to two synthetic standards; the equivalent of 0.2% iron oxide was added. The net effect of these additions on the emission intensity is zero.

The result of additions of sulfate to the synthetic standards is shown in Table III; the equivalent of 0.5% sulfur trioxide was added. There is a depression of approximately 3% in both the calcium and magnesium readings with no perchloric acid present, but no effect on the emission intensity with perchloric acid present. It seems that a sufficient excess of perchlorate anion is present at a concentration of 0.0125*N* to exhibit a predominating effect over that of the sulfate and iron ions, as has been suggested by Baker and Johnson (*1*).

To determine whether the effect of perchlorate on the calcium and magnesium emission intensity in the standard reaches a maximum value, after which it levels off, it was decided to test the intensity of the unknown by adding a large excess of perchloric acid to various typical glass decompositions. In Table IV under Unknown, the column headed 0.0625*N* perchloric acid represents the typical glass decomposition and the column headed 0.125*N* perchloric acid represents an addition of a 100% excess concentration of perchloric acid. Evidently the glass solutions obtained by the procedure for glass analysis have enough perchloric acid in them to exhibit a limiting effect. The standards also show no increase in emission intensity of calcium and magnesium on addition of a 100% excess of perchloric acid over that shown in Figure 1.

If the procedure for glass is to be extended to other materials, the amount of perchlorate in the residue of the perchloric-hydrofluoric decomposition, as illustrated in Table V, must be studied. The procedure used in determining the perchlorate here was Scott's ammonium chloride sublimation (*8*).

Boron was found to depress the calcium readings in the synthetic standards. In an experiment, 25 mg. per liter of boric oxide as boric acid, equivalent to 0.5% boric oxide in the sample, was added to the E_1, E_3, L synthetic standard; the calcium emission was found to be depressed some 10%. The boron need not be considered when the sample is brought into solution by perchloric-hydrofluoric decomposition, however.

Phosphorus was found to lower the calcium emission decidedly in one synthetic standard in which it was included as phosphoric acid, with perchloric acid absent. Addition of perchloric acid to about 0.05*N* raised the calcium emission to an extent even greater than 12%. This phenomenon was not studied any further, because there was apparently no perchlorate in the residue of this phosphate glass perchloric-hydrofluoric decomposition. Instead of perchloric acid, phosphoric acid was added to the standard for this glass, in an amount equivalent to the phosphorus in the glass; the calcium determinations by the flame then checked closely the standard titrimetric results.

Potassium interferes with the manganese emission somewhat, because of the weak potassium emission line at 404 mμ. When the potassium concentration becomes greater than 10 times the manganese concentration, the interference begins to become serious. The manganese oxide blank for the Sa synthetic standard, which standard contains the equivalent of 0.2% manganese oxide and 1.2% potassium oxide, definitely shows a peak at 404 mμ which is due to the potassium in the solution. However, the manganese peak in this particular standard is over and above the potassium at 404 mμ; the manganese peak at 403 mμ, when present strongly, "blots out" the potassium at 404 mμ with the Beckman Model B. An instrument with slightly greater resolving power than the Model B could separate these two peaks.

Manganese interferes with the magnesium emission at 383 mμ by raising the background and, if present in widely divergent amounts in the standard and the unknown, the determinations on magnesium are erroneous.

The amount of emission intensity of each component of the standard, which is due to background radiation, is illustrated graphically for two different standards in Figure 2, *A* and *B*.

Table III. Effect of Sulfate Salts on Calcium and Magnesium Readings of Synthetic Standard

Standard	Component Measured	SO_3, Mg./L. None	SO_3, Mg./L. 25.0	Net Effect of Additive, %
		Intensity Readings		
		0.0125*N* $HClO_4$		
E_1, E_3, L	CaO	60.0	60.0	0.0
	MgO	58.0	58.0	0.0
S_1, S_2	CaO	58.0	58.0	0.0
	MgO	56.0	56.0	0.0
		No $HClO_4$		
E_1, E_3, L	CaO	64.0	62.0	−3.1
	MgO	63.5	61.5	−3.2
S_1, S_2	CaO	64.0	62.5	−2.4
	MgO	63.5	61.5	−3.2

Table IV. Effect of Perchloric Acid Additions to Synthetic Standard and Unknown Solutions

Solution Being Read Standard	Component Measured	Intensity Readings 0.0625*N* $HClO_4$	Intensity Readings 0.125*N* $HClO_4$	Net Effect, %
E_1, E_3, L	CaO	65.0	65.0	0.0
	MgO	66.0	66.0	0.0
S_1, S_2	CaO	65.0	65.0	0.0
	MgO	66.0	66.0	0.0
Unknown				
E_1	CaO	60.0	60.0	0.0
	MgO	62.0	62.0	0.0
E_2	CaO	60.0	60.0	0.0
	MgO	62.0	62.0	0.0
S_2	CaO	61.0	61.0	0.0
	MgO	62.0	62.0	0.0
L	CaO	61.0	61.0	0.0
	MgO	63.0	63.0	0.0
Sa	CaO	61.0	61.0	0.0
	MgO	63.0	63.0	0.0

Figure 2, *A*, shows a high background effect for manganese present in very low concentration in the standard, an intermediate background effect for magnesium present in intermediate concentration, and an even lower background for potassium present in much lower concentration than magnesium. Sodium and calcium show a negligible background. In Figure 2, comparing *A* to *B* illustrates the change in background with the change in concentration of a given element. Here the magnesium emission becomes a tenth of that illustrated in Figure 2, *A*, and is impractical to measure with this instrument, the background effect being 95% of the emission at full scale and the slope of background *vs.* standard emission approaching 45°. Doubling the manganese concentration results in a nice lowering of the background for that element and sodium, potassium, and calcium backgrounds here are negligible.

PREPARATION OF GLASS STANDARDS

Aliquots of the standards containing a single chloride salt were pipetted in order to make the composite solutions designated as synthetic standards. The known composition of the glass or glasses to be analyzed is used in calculating the amount of each component in 1 liter of the synthetic standard at the level of concentration of 0.5 gram of glass per 100 ml. of solution. The calculations are simply set up as in Table VI.

This standard is used for a range of concentrations as follows:

Al_2O_3	1.6 to 2.1%
CaO	6.8 to 8.6
MgO	2.6 to 3.5
Na_2O	13.6 to 14.9
K_2O	0.16 to 0.32
MnO	0.00 to 0.10

The glasses for which the standard illustrated is used also contain silica, ferric oxide, barium oxide, sulfate, fluorine, arsenic pentoxide, and boric oxide. Aluminum is included, but iron,

Table V. Perchlorate Present in Hydrofluoric-Perchloric Residues

Material	Weight, G.	Perchlorate, G.	Chloride
Glass	0.1250	0.07	Negative
	0.5000	0.25	Negative
Slag	0.5000	1.0	Negative

Table VI. Typical Glass Standard Composition[a]

(E_1, E_3, L synthetic standard, prepared 6-7-54)

Component	% in Theoretical Glass in Standard (*C*)	Mg. per Liter of Synthetic Standard
Al_2O_3	2.00	100.00
CaO	8.00	400.00
MgO	3.00	150.00
Na_2O	14.00	700.00
K_2O	0.20	10.00
MnO	0.050	2.50

[a] Plus 4.0 ml. of 72% perchloric acid per liter.

barium, and sulfate are not included for the reasons developed in the experimental section of this paper. Silica, arsenic pentoxide, boric oxide, and fluorine are volatilized in the course of the perchloric-hydrofluoric disintegration when the remaining elements are also changed to their perchlorate salts. The theoretical equivalent of perchloric acid in the residue of 0.5000 gram of these particular glasses is approximately 0.28 gram agreeing with experimental determinations of perchlorate on the residue. This is an amount greater than that needed for a maximum enhancement of the calcium and magnesium emission. In order to achieve the same effect in the standard, 4.0 ml. of 72% perchloric acid must be added per liter. In the case of a phosphate glass, a perchlorate assay of the residue from the disintegration of the particular glass in question indicates the steps to be taken in making the synthetic standard.

The blanks are made up exactly the same as the synthetic standards, except for omission of the component for which the background effect is being measured.

PROCEDURE FOR GLASS ANALYSIS

Perchloric-Hydrofluoric Decomposition. Weigh out a 0.5000-gram sample of powdered glass (200-mesh or finer) on a damped balance and transfer to a 60-ml. platinum evaporating dish. Add 7 ml. of perchloric acid and 5 ml. of hydrofluoric acid. Grasping the dish with platinum-tipped tongs, swirl the contents to mix and set on the edge of an asbestos-covered hot plate. When the effervescing dies down, move the dish more to the center of the hot plate and take down to first dense white fumes of perchloric acid. Remove from the hot plate and cool the platinum in a small water bath, and when cool examine for undecomposed silicates. If the material is not completely dissolved at this point, add 1 to 2 ml. more of hydrofluoric acid and repeat the evaporation to perchloric fumes. As soon as the material is completely decomposed, take down to dryness. Cool the dish in water again, remove from the water bath, and add 1 to 2 ml. of 6*N* hydrochloric acid and hot water to dissolve. Transfer to a 100-ml. volumetric flask, dilute to volume, and mix. The solution should be completely clear at this point.

An alternative procedure is to decompose a 0.1250-gram sample and make up to a final volume of 25 ml.; in either case, the final concentration of sample is the same. The smaller sample uses less time and materials in the decomposition with still enough solution for the determination of sodium, potassium, calcium, magnesium, and manganese. A 1 to 40 dilution of this solution is made for taking the sodium readings, which are read against a 1 to 40 dilution of the synthetic standard. The dilution is best made by transferring 5 ml. of the more concentrated solution to a 200-ml. volumetric flask and diluting to volume.

Flame Photometric Measurements. The gas pressures employed are 4 pounds per square inch of hydrogen and 10 pounds per square inch of oxygen for sodium, potassium, calcium, and manganese; for very weak concentrations of the preceding elements and for magnesium, 5 pounds per square inch of hydrogen and 10 pounds per square inch of oxygen are used. As a general rule, the sensitivity control on the instrument and the phototube voltage are set sufficiently high to get the narrowest possible slit opening, but not below 0.01 mm. Sodium is read at 589 mμ, potassium at 767, calcium at 422.7, manganese at 403, and magnesium at either 383 or 371 mμ. After the desired emission peak is located exactly, the wave-length setting is not disturbed until all of the determinations on that particular element are accomplished. All solutions to be analyzed may be lined up in 5-ml. beakers covered with small watch glasses to minimize concentration of solute by evaporation. The wave length having been set, the slit is adjusted so that the galvanometer response is around 60 to 80% transmittance; the slit is then not changed while a given sample is read. Greater accuracy of the readings is obtained the higher up the per cent transmittance scale the readings can be taken; however, the needle fluctuation becomes greater the higher up the scale one goes. With the particular instrument used in this work, 60 to 80% transmittance was a likely compromise between accuracy of the reading and the needle fluctuation.

For the most precise work, the synthetic standard solution is read coincidentally. After a stable reading of the standard in the flame is obtained, the standard is immediately removed, the aspirator is given a 1 to 2-second rinse with distilled water, and the unknown is placed in the flame. After a stable reading of the unknown is obtained, the capillary is rinsed and the standard measured again to check the first reading. If the two standard readings do not agree, the unknown reading is repeated, followed by another reading of the standard, and so on, until the standard readings before and after the unknown reading agree within ± 0.5% transmittance. The capillary is always rinsed for 1 to 2 seconds with distilled water between readings, and the dark current is zeroed while doing this. A blank is read in conjunction with each determination, or the value for any given determination is read from a prepared curve, such as Figure 2.

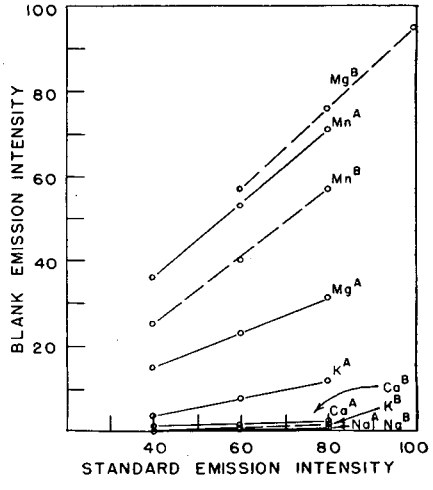

Figure 2. Typical background readings of synthetic standards

A. E_1, E_3, L synthetic standard composition (see Table VI)

B. Sa synthetic standard composition, milligrams per liter

Al_2O_3	130.0	Na_2O	700.0
CaO	500.0	K_2O	60.0
MgO	15.0	MnO	5.0

In calculating the results, use is made of the fact that, in the case of the usual glass and in a sufficiently dilute solution, there is a straight-line relationship between the emission intensity and the element concentration. It was found in numerous experi-

ments in the laboratory that, in the case of sodium, potassium, calcium, magnesium, and manganese, the straight line is fitted very closely by the following equation over a relative deviation from the standard concentration of at least ± 25%.

$$V = \frac{U - B}{S - B} \times C$$

where U = reading of sample
S = reading of standard
B = reading of blank
C = per cent of component being measured which is present in the synthetic material in the standard
V = per cent of component being measured which is present in the unknown sample

The background effect is compensated for by the blank. All other interferences are incorporated in the standard. These effects include line spectral interference, stray light, band spectral interference, continuum interference (including anionic effect), and temperature and viscosity effects.

DISCUSSION OF RESULTS

A single scale reading on the Model B cannot be read closer than 0.5% transmittance when the flame is used, but the average of three or more readings gives a result accurate to about 0.2 division. When the readings are taken at 80% transmittance a photometric accuracy of 2.5 parts in a thousand is given. The accuracy is less when the blank is greater than zero, becomes progressively less with increasing background, decreases with lower scale readings, and increases with higher scale readings of the sample. Almost always precision has been found to be within photometric accuracy.

A standard glass which has been analyzed in this laboratory a number of times by a wet-analysis procedure, was used to compare the wet-analysis method to the proposed procedure. The results in Table VII show good agreement with sodium oxide, potassium oxide, and manganese oxide determinations, but low calcium oxide and high magnesium oxide results. In order to check the consistency of the discrepancy between the two methods, the calcium oxide content of eight different glasses was determined on many occasions by the flame and by the wet-analysis method. The flame results were consistently below the wet analysis by 0.2 to 0.3% in seven out of the eight glasses; in the eighth case, the flame results were higher than the wet analysis, and in this case the glass was also very low in magnesium oxide.

Table VII. Comparison of Flame Analysis of Standard Glass with Wet Analysis Procedure

Component	Analysis, %		No. of Detns.
	Wet	Flame	
CaO	8.19	7.98	4
MgO	3.07	3.32	4
Na_2O	14.27	14.25	6
K_2O	0.24	0.25	6
MnO	0.02	0.02	5

Investigation of the calcium oxalate precipitate in the wet-analysis method showed coprecipitation of some magnesium oxalate and thereby explained the discrepancy in the results by the two methods, as the filtrate from the calcium oxalate precipitation had been used for the magnesium precipitation.

The calcium oxalate precipitate was tested by dissolving it in dilute hydrochloric acid and examining for peaks of magnesium on the flame spectrophotometer. Reagent grade calcium chloride was dissolved and precipitated as the oxalate by the wet-analysis procedure used for the glass, and this precipitate was examined in the same manner on the flame spectrophotometer. Definite peaks at 383 and 371 mμ were observed for the precipitate from the glass analysis, whereas no peaks at all in this region showed up in the reagent precipitate.

To test this explanation further, Bureau of Standards glass No. 80 was analyzed for sodium oxide, potassium oxide, calcium oxide, and magnesium oxide by the flame procedure. The results (Table VIII) show close agreement in all cases between the flame analysis results and the bureau's certified values.

Table VIII. Flame Analysis of National Bureau of Standards Glass Sample 80

Component	Certified Value, %	Flame Analysis, %	
		Sample A	Sample B
CaO	4.65	4.57	4.60
MgO	3.23	3.26	3.26
Na_2O	16.65	16.62	16.62
K_2O	0.04	0.039	0.038

Table IX. Comparison of Standard Glass Flame Analysis with Results of Other Laboratories

Component	Flame Analysis, %	Laboratory A,[a] %	Laboratory B,[b] %	Laboratory C,[c] %
CaO	7.98	8.03	8.08	7.64
MgO	3.32	3.20	3.40	3.20
Na_2O	14.25	14.30	14.12	14.30
K_2O	0.25	0.22	0.19	0.37
MnO	0.02			

[a] Hartford-Empire Co. Testing Laboratory.
[b] Sharp-Schurtz Co. Testing Laboratories.
[c] Solvay Process Division Laboratories.

In general, analytical results often vary from one procedure to another, but the above account is a good illustration of how selective flame photometric analysis can be, and how the flame photometer can be used as a tool in a rapid check of gravimetric precipitates for purity. Further comparison of results with independent testing laboratories is shown in Table IX, where it is apparent that the results of laboratories A, B, and C all fall within the range obtained by this method.

EXTENT OF APPLICATION

Sand, slag, and feldspar have been analyzed successfully by applying the procedure outlined. A further technique which may be used with many materials, especially with minerals, is to dissolve a standard sample of the material to be analyzed, such as National Bureau of Standards dolomite or magnesite, compare the flame emission of the standard with the unknown solution, and use the certified analysis values for calculation of the unknown sample. The background effect may be measured very closely in these cases by taking readings just before and just after the emission peak, where the intensity levels off and there are no immediately adjacent emission peaks. In the case of broad oxide bands, however, the technique of estimating the background is not so simple.

Sodium may be determined in materials such as salt cake and soda ash by comparing flame intensities of their solutions at the proper dilution with similar solutions of the analyzed laboratory reagents—in this case sodium sulfate and sodium carbonate, respectively.

Other materials which have been analyzed successfully by this method include nepheline syenite, razorite, and cryolite.

In analyzing materials, such as aluminum silicates, that cannot be readily dissolved by the perchloric-hydrofluoric disintegration method used in the glass analysis procedure, the following modification is often found to work well.

Enough hydrofluoric acid alone is added to the sample to volatilize all the silica and boron. The sample is then cautiously

taken down to dryness on a medium hot plate and cooled. To the platinum dish are added, by running down the sides, about 5 ml. of perchloric acid and 2 ml of hydrofluoric acid. The dish is swirled to mix, and again taken down to dryness and cooled. The residue should now yield to solution in dilute hydrochloric acid.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Baker, G. L., and Johnson, L. H., ANAL. CHEM. 26, 465 (1954).
(2) Beckman Instruments, Inc., South Pasadena, Calif., Bull. 278, May 1952.
(3) Brabson, J. A., and Wilhide, W. D., ANAL. CHEM. 26, 1060 (1954).
(4) Close, P., Smith, W. E., and Watson, M. T., Jr., *Ibid.*, 25, 1022 (1953).
(5) Close, P., and Watson, M. T., Jr., *J. Am. Ceram. Soc.* 37, 235 (1954).
(6) Gilbert, P. T., Jr., Hawes, R. C., and Beckman, A. O., ANAL. CHEM. 22, 772 (1950).
(7) Mosher, R. E., Bird, E. J., and Boyle, A. J., *Ibid.*, 5, 715 (1950).
(8) Scott, W. W., "Standard Methods of Chemical Analysis," 5th ed., p. 275, Van Nostrand, New York, 1945.

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Colorimetric Determination of Cycloserine, a New Antibiotic

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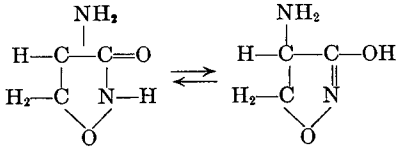
A specific method of analysis was needed for cycloserine, a new antibiotic, as an aid in chemical evaluation. Cycloserine reacts with sodium nitrotopentacyanoferroate in slightly acidic medium to give an intense blue-colored complex suitable for quantitative measurement at 625 mμ. The color deviates slightly from Beer's law but is reproducible in the range of 5 to 200 γ of cycloserine. The test quantitatively determines the antibiotic in amounts as little as 2 to 3 γ and has an accuracy within +2% and a precision within ±1%. The method is specific for the cycloserine molecule and has been adapted to all phases of its production and use. The results are in good agreement with the bioassay.

CYCLOSERINE is the generic name of an antibiotic isolated from a culture of *Streptomyces orchidaceus* by Harned, Hidy, and LaBaw (*3*). Preliminary clinical evaluation has demonstrated its effectiveness in pulmonary tuberculosis (*2*) and certain genitourinary infections (*9*). Cycloserine is a cyclic hydroxamic acid derivative of serine, determined to be D-4-amino-3-isoxazolidinone (*1, 4, 5, 7, 8*), and has the formula:



A specific method of analysis was needed for cycloserine in the presence of amino acids, other antibiotics, degradation products, and compounds of biological origin. A micromethod most suited the several requirements. Therefore, colorimetric methods were examined, because these are the most universally applicable.

Cycloserine reacts with sodium nitritopentacyanoferroate in slightly acidic medium to give an intense blue-colored complex suitable for quantitative measurement at 625 mμ. The color develops rapidly and is stable for several hours. It deviates slightly from Beer's law but is reproducible in the range of 5 to 200 γ of cycloserine.

The method is specific and sensitive for the cycloserine molecule and has been adapted to all phases of its production and use. The results are in good agreement with the bioassay.

APPARATUS

SPECTROPHOTOMETER, Beckman Model DU, with 1-cm. Corex cells

CENTRIFUGE, any laboratory type for 15-ml. tube

VACUUM OVEN, 60° C.

WEIGHING BOTTLE, glass-stoppered

DESICCATOR, with phosphorus pentoxide desiccant

PIPETS, 1.0, 2.0, 3.0, 4.0, and 10.0 ml. Normax brand or equivalent

BURET, 50 ml., Normax brand or equivalent

REAGENTS

CYCLOSERINE STANDARD. Repeated biological assay, elemental analyses, optical rotation, titration, and other tests indicate that the purity approaches 100%.

SODIUM HYDROXIDE, 4.000 and 0.100*N* solutions

ACETIC ACID, 3.000 and 1.000*N* solutions

SULFURIC ACID, 0.666*N* solution

SODIUM TUNGSTATE, Na_2WO_4, 10 ± 0.5% aqueous solution

TUNGSTIC ACID REAGENT. Mix equal volumes of the sodium tungstate and sulfuric acid solutions. This reagent must be made fresh daily.

SODIUM NITROPRUSSIDE, $Na_2Fe(CN)_5NO.2H_2O$. Prepare a fresh 4 ± 0.5% aqueous solution every 2 weeks and store in a glass-stoppered brown bottle.

CYCLOSERINE COLOR REAGENT, $Na_4[Fe(CN)_5NO_2]$. Mix equal volumes of the sodium nitroprusside and 4*N* sodium hydroxide solutions just prior to use. The reagent is ready for immediate use and must be discarded after one set of determinations because of instability.

ACTIVATED CHARCOAL, Darco G-60 or equivalent

PREPARATION OF CALIBRATION CURVE

Add 0.10 to 0.12 grams of the crystalline cycloserine to a tared glass-stoppered weighing bottle and place in the 60° C. oven at 10 to 15 mm. of mercury pressure for 2 hours. Cool in the desiccator.

Prepare a solution of cycloserine standard in 0.100*N* sodium hydroxide solution to contain 1.0 mg. per ml. (This solution is stable for several weeks if kept refrigerated.) Transfer by means of a buret, 0.0-, 2.5-, 5.0-, 7.5-, 10.0-, 12.5-, 15.0- 17.5-, and 20.0-ml. portions of the standard solution to separate 100-ml. volumetric flasks. Dilute each to volume with 0.100*N* sodium hydroxide.

Transfer 1.0 ml. of each dilution into a test tube. Add 3.0 ml. of 1.000*N* acetic acid and 1.0 ml. of color reagent and mix. Allow the solution to stand at room temperature for 10 minutes. Transfer to a 1-cm. Corex cell and read the absorbance at 625 mμ, using the solution containing 0.0 ml. of standard as the blank.

Plot concentration against absorbance on linear graph paper. The curve deviates slightly from a straight line. The above standards equal 0, 25, 50, 75, 100, 125, 150, 175 and 200 γ of cycloserine, respectively.

DETERMINATION

The determination is the same as the calibration, except the sample is prepared so that a 1.0-ml. aliquot of 0.100*N* sodium hydroxide solution does not contain more than 150γ of cyclo-

serine per milliliter. If the color is too dark to read, the analysis must be repeated, using a smaller aliquot. The colored complex cannot be diluted.

APPLICATIONS

This method has been applied successfully to the determination of crystalline cycloserine and its salts in pharmaceutical preparations, fermentation, and process samples, and in blood, urine, cerebrospinal fluid, and other biological fluids.

DETERMINATION OF CRYSTALLINE CYCLOSERINE

Weigh accurately 0.10 to 0.12 gram of dry, crystalline cycloserine, transfer quantitatively to a 100-ml. volumetric flask, and dilute to volume with 0.100*N* sodium hydroxide.

Transfer 10 ml. of this solution to a second 100-ml. volumetric flask, and dilute to volume with 0.100*N* sodium hydroxide.

Analyze a 1.0-ml. aliquot of this solution for cycloserine, using for a blank 1.0 ml. of 0.100*N* sodium hydroxide solution treated in the same manner as the sample.

Typical results on the determination of crystalline cycloserine by the color method as compared to a biological assay are given in Table I.

CYCLOSERINE IN BLOOD PLASMA

Pipet 2.0 ml. of plasma to a 15-ml. centrifuge tube, add 4.0 ml. of the tungstic acid reagent, mix well, and centrifuge for several minutes until a clear filtrate is obtained.

Pipet 3.0 ml. of the clear filtrate (this is equivalent to 1.0 ml. of plasma) to a test tube. Add 1.0 ml. of 3.000*N* acetic acid, and 1.0 ml. of the freshly prepared color reagent and mix. Allow the reaction to stand at room temperature for 10 minutes. Transfer to a 1-cm. Corex cell and read the absorbance at 625 mμ, using a blank solution prepared from 1.0 ml. of 0.100*N* sodium hydroxide and reagents to set the spectrophotometer.

Data for the determination of cycloserine in blood plasma are:

Added, γ/Ml.	Found, γ/Ml.
10	10
50	49
100	99
200	201

CYCLOSERINE IN CEREBROSPINAL FLUID

Normal cerebrospinal fluid can be analyzed for cycloserine without preliminary treatment.

Pipet 1.0 to 3.0 ml. of clear cerebrospinal fluid to a test tube. If necessary, add water to make 3.0 ml. Add 1.0 ml. of 3.000*N* acetic acid and 1.0 ml. of color reagent and proceed as in above procedures, using a blank prepared from 1.0 ml. of 0.100*N* sodium hydroxide and reagents to set the spectrophotometer.

Data for the determination of cycloserine in cerebrospinal fluid are:

Added, γ/Ml.	Found, γ/Ml.
5	5.0
10	9.9
50	50.1
100	100

CYCLOSERINE IN URINE

Dilute the urine so that a milliliter aliquot contains between 50 and 150 γ of cycloserine per milliliter and then analyze. In cases where pigmentation is high and cycloserine content is low the urine should be neutralized and decolorized with char before analysis. Analyze a 1 to 3-ml. aliquot, using 1.0 ml. of 0.100*N* sodium hydroxide and the reagents as a blank.

Data for the determination of cycloserine in urine are given below. The low recoveries of known amounts of cycloserine from urine indicate the presence of some interference.

Added, γ/Ml.	Found, γ/Ml.	Added, γ/Ml.	Found, γ/Ml.
100	94.0	100	96.0
100	97.0	100	87.5
100	93.0	100	97.0

CYCLOSERINE IN FERMENTATION MEDIA

Fermentation media and process recovery samples can be analyzed for cycloserine after filtration and dilution. If pigmentation is high and the cycloserine titre is low, the broth should be decolorized with char before analysis. Analyze a 1 to 3-ml. aliquot, using 1.0 ml. of 0.100*N* sodium hydroxide and the reagents as a blank.

Table I. Determination of Crystalline Cycloserine

(microgram per milligram)

Lot No.	Theory	Color Assay	Biological Assay[a]
1	1000	988.5	1031
2	1000	990.2	1103
3	1000	988.7	1033
4	1000	1000.0	1005

[a] Plate assay method using *Micrococcus pyogenes* var. *aureus* ATCC #6538P.

Table II. Acid-Base Effect on Color

Vol. 4.00*N* Acetic Acid, Ml.	Absorbance 100 γ of Cycloserine	Vol. 4.00*N* Acetic Acid, Ml.	Absorbance 100γ of Cycloserine
0.50	0.000	1.20	0.601
0.60	0.590	1.40	0.600
0.70	0.600	1.60	0.590
0.80	0.600	1.80	0.575
0.90	0.599	2.00	0.540
1.00	0.600		

EXPERIMENTAL

The effects of several variables were investigated for their influence on the color formation and quantitative application of the reaction. The factors included the absorbtion curve, stability and intensity of color, time and temperature of reaction, stability and concentration of sodium nitritopentacyanoferroate, acid-base concentration, conformity to Beer's law, and interference from other compounds. A Beckman Model DU spectrophotometer was used.

REAGENT PREPARATION

When sodium nitroprusside is treated at room temperature with a strong aqueous solution of sodium hydroxide, an iron(II) compound is formed in which the nitroso group is oxidized by iron(III) to the nitro group (*6*), and sodium nitritopentacyanoferroate, $Na_4[Fe^{II}(CN)_5(NO_2)]$, is formed. This reagent is easily oxidized by light and oxygen, and the oxidized reagent does not give the characteristic color with cycloserine. Fresh reagent must be prepared for each set of determinations.

For convenience, two stock solutions of 4.0*N* sodium hydroxide and 4% aqueous sodium nitroprusside are prepared and equal portions are mixed for each analysis of cycloserine. The reagent must be used within 15 minutes after preparation.

ABSORPTION SPECTRUM

The absorbance curve of the blue complex of cycloserine and sodium nitritopentacyanoferroate shows a maximum absorbance at 625 mμ.

TRANSMITTANCE AND CONCENTRATION

Calibration curves were determined for the cycloserine–sodium nitritopentacyanoferroate by plotting absorbance against concentration on linear graph paper. The color deviates slightly from Beer's law in the range of 10 to 200 γ of cycloserine. The curve is reproducible at all points when a standardized procedure is followed.

COLOR STABILITY

Cycloserine was treated to develop the colored complex. The absorbances of the samples were determined at various time intervals. The color was found to be stable for at least 4 hours.

TIME AND TEMPERATURE OF REACTION

The color complex was prepared and spectrophotometer readings were made intermittently in order to determine the required

amount of time for complete color development. A development time of 10 minutes was chosen as optimum to ensure complete color intensity.

The complex between cycloserine and sodium nitritopentacyanoferroate was found to be sensitive toward temperature changes. Although the color develops rapidly at room temperature, the highest precision and reproducibility were obtained in a temperature-controlled laboratory where the temperature was 25° ± 1° C. and the relative humidity was 50 ± 2%. Cooling samples below 15°C. before color development resulted in slow color formation, and heating samples above 50°C. completely destroyed the color.

REAGENT CONCENTRATION

The concentration of reagent is not critical, as long as an excess amount is added. A 2% solution of sodium nitritopentacyanoferroate in 2.000*N* sodium hydroxide gives a low reagent blank and maximum color.

ACID-BASE RELATION TO COLOR

The complex develops in weakly acidic medium. Acetic or phosphoric acids can be used, but stronger acids such as sulfuric, hydrochloric, or nitric destroy the color complex.

The optimum amount of acid required was investigated. Several aliquots containing 100 γ of cycloserine were treated with 1.0 ml. of color reagent in 2.0*N* alkali. The amount of 4.0*N* acetic and the water content were varied. The total volume was held constant at 5.0 ml. The results are given in Table II.

Three milliliters of a 1.000*N* solution of acetic acid was chosen as optimum when 1.0 ml. of color reagent equivalent to 2.000*N* alkali was used.

INTERFERENCE

In order to determine the specificity of the nitritopentacyanoferroate method toward cycloserine several compounds were tested. These included degradation compounds, amino acids and other antibiotics. The compounds tested were: D-serine, serine amide, serine hydroxamic acid, hydroxylamine hydrochloride, D-threonine, L-tyrosine, D-valine, L-proline, L-hydroxyproline, D-lysine, D-glutamic acid, glycine, β-alanine, N-methylglucamine, L-histidine, L-cysteine, asparagine, nicotinamide, uric acid, methionine, glucurono-lactone, rutin, urea, benzylpenicillin, streptomycin, dihydrostreptomycin, bacitracin, Aureomycin, Terramycin, neomycin, glucose, lactose, and sucrose. These compounds did not give a blue color.

A positive reaction was given by derivatives of cycloserine which still retain the basic ring structure of cycloserine. The derivatives prepared in the laboratory and tested were: monoacetyl, monobenzoyl, mono-and diisocyanate, and desaminocycloserine.

The test appears to be specific for the determination of the cycloserine molecule in a wide variety of samples.

SUMMARY

A colorimetric procedure for the specific determination of cycloserine, a new antibiotic, has been presented. The proposed test quantitatively determines the antibiotic in amounts as little as 2 to 3 γ and has an accuracy within ± 2% with a precision within ± 1%. The method has been adapted to the determination of crystalline cycloserine and its salts in pharmaceutical preparations, blood plasma, urine, cerebrospinal fluid, other biological fluids, fermentation broths, and all phases of processing. Interference has been encountered only in urine. A close correlation has been found between a biological assay and the color assay.

LITERATURE CITED

(1) Cuckler, A. C., Frost, B. M., McClelland, L., and Solotorovsky, M., *Antibiotics & Chemotherapy* **5**, 191 (1955).
(2) Epstein, I. G., Nair, K. G. S., and Boyd, L. J., *Antibiotic Med.* **1**, 80 (1955).
(3) Harned, R. L., Hidy, P. H., and LaBaw, E. K., *Antibiotic & Chemotherapy* **5**, 204 (1955).
(4) Harris, D. A., Ruger, M., and others, *Ibid.*, **5**, 183 (1955).
(5) Hidy, P. H., Hodge, E. B., and others, *J. Am. Chem. Soc.* **77**, 2345 (1955).
(6) Hofman, K. A. H., *Ann. Chem., Justus Liebigs* **312**, 1 (1900); *Chem. Zentr.*, 557–9 **1900** *II*.
(7) Kuehl, F. A., Jr., Wolf, F. J., and others, *J. Am. Chem. Soc.* **77**, 2344 (1955).
(8) Stammer, C. H., Wilson, A. N., Holly, F. W., and Folkers, K., *Ibid.*, **77**, 2346 (1955).
(9) Welch, H., Putman, L. E., and Randall, W. A., *Antibiotic Med.* **1**, 72 (1955).

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Color Reaction of Salicylic Acid and Nitrite with Cupric Ion

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The variables which affect the determination of copper by the Jorissen reaction (the formation of a red color upon heating a solution containing salicylate, nitrite, and cupric ion) have been investigated. The pH of the solution, the concentration of nitrite and of salicylate, and the duration of the heating period must be controlled if this reaction is to yield reliable results. Under the conditions suggested as a result of this study, the recommended copper concentration range is about 2 to 9 p.p.m. At the optimal concentration (about 5 p.p.m.) the standard deviation is about 0.04 p.p.m. Although a detailed study of interferences has not been made, the method appears to be fairly selective for copper. Information regarding the identity of the red reaction product is also presented.

THE red color formed upon heating a solution containing salicylic acid, inorganic nitrite, and cupric ion has been known for many years. It was first reported by Jorissen (*2*) and later studied by Schott (*4*) and Sherman and Gross (*5*). More recently, this color reaction has been mentioned by Müller and Burtsell (*3*) and has found its way into several standard treatises (*1*, *6*, *7*) where it is suggested as the basis of a colorimetric copper determination and also as a confirmatory test for salicylates. In spite of this attention, the reaction has never been studied critically to establish optimal conditions for analytical procedures, nor has the nature of the red reaction product been elucidated.

In the present investigation, it has been found that the variables affecting the development of the red color must be controlled much more carefully than previous reports have in-

dicated in order to obtain reproducible results.* Conditions are suggested under which it is possible to determine copper in concentrations from about 0.2 to 15 p.p.m. The most satisfactory concentration range extends from about 2 to 9 p.p.m.. with an optimal concentration of about 5 p.p.m. which can be determined with a standard deviation of about 0.04 p.p.m. The method seems fairly selective in that only relatively large quantities of several other metals imitate the copper reaction. The available data shed considerable light upon the nature of the reaction, but definitive identification of the red substance has proved difficult.

Table I. Reproducibility at Three Concentrations

	2.06 P.P.M. Cu		5.15 P.P.M. Cu		10.3 P.P.M. Cu	
	Absorbance *vs.* blank	Dev. from mean absorbance	Absorbance *vs.* blank	Dev. from mean absorbance	Absorbance *vs.* blank	Dev. from mean absorbance
	0.183	0.006	0.456	0.000	0.872	0.014
	0.183	0.006	0.459	0.003	0.850	0.008
	0.177	0.000	0.462	0.006	0.855	0.003
	0.176	0.001	0.456	0.000	0.855	0.003
	0.177	0.000	0.452	0.004	0.870	0.012
	0.177	0.000	0.455	0.001	0.858	0.000
	0.176	0.001	0.453	0.003	0.868	0.010
	0.175	0.002	0.452	0.004	0.845	0.013
	0.175	0.002	0.453	0.003	0.848	0.010
	0.175	0.002	0.453	0.003	0.859	0.001
	0.183	0.006	0.457	0.001	0.862	0.004
	0.177	0.000	0.458	0.002	0.862	0.004
	0.176	0.001	0.457	0.001	0.838	0.020
	0.173	0.004	0.463	0.007	0.850	0.008
	0.177	0.000	0.457	0.001	0.848	0.010
	0.177	0.000	0.464	0.008	0.865	0.007
	0.173	0.004	0.454	0.002	0.870	0.012
	0.175	0.002	0.456	0.000	0.861	0.003
	0.177	0.000	0.454	0.002	0.857	0.001
	0.178	0.001			0.862	0.004
Range	Absorbance 0.173–0.183	Cu, p.p.m. 2.02–2.13	Absorbance 0.452–0.464	Cu, p.p.m. 5.11–5.24	Absorbance 0.838–0.872	Cu, p.p.m. 10.1–10.5
Median	0.177	2.06	0.456	5.15	0.858	10.3
Mean	0.177	2.06	0.456	5.15	0.858	10.3
Av. dev.	0.0019	0.022	0.0027	0.031	0.007	0.085
Std. dev.	0.0029	0.034	0.0036	0.041	0.009	0.11

VARIABLES AFFECTING COLOR DEVELOPMENT

Apparatus and Reagents. All absorbance measurements were performed with a Beckman Model DU spectrophotometer, using 1-cm. Corex cells. Measurements of pH were performed with a Beckman Model G pH meter. Reagent grade materials were used throughout the study. The following solutions are employed in the method.

STANDARD CUPRIC NITRATE, about 20γ of copper per milliliter, prepared from electrolytic copper foil

SODIUM SALICYLATE, $1.0 \times 10^{-2}M$

ACETATE BUFFER, $1M$, adjusted to pH 4.2

POTASSIUM NITRITE, 50 mg. per ml., freshly prepared

Absorption Spectra. Figure 1 shows that the red solutions exhibit an absorption maximum at about 520 mμ. The band is sufficiently broad so that the wave-length setting is not critical. All measurements reported below were performed at this wave length. (The ultraviolet region of the spectrum was also investigated: Although a prominent absorption maximum was found, it was almost equally strong in both blanks and copper solutions.)

Effect of pH. A series of solutions was prepared, each containing 60γ of copper, 2 ml. of a $10^{-3}M$ sodium salicylate solution, 1 ml. of potassium nitrite solution (50 mg. of potassium nitrite per ml.), and acetic acid to a final concentration of $0.1M$. The pH values of the solutions were adjusted with sodium hydroxide or hydrochloric acid as desired, and the solutions were brought to a final volume of 20 ml. A similar series of "blanks," in which the copper was omitted, was prepared. The solutions were heated for 1 hour in a water bath at about 80° C., cooled to room temperature, and measured at 520 mμ against a distilled water reference.

The results of this experiment, clearly showing the very critical necessity for pH control, are plotted in Figure 2. The optimal pH is about 4.1 to 4.2. The unfortunate need for very careful buffering represents one of the serious drawbacks in the use of this method.

Effect of Salicylate Concentration. Varying quantities of the $10^{-2}M$ sodium salicylate solution were added to a series of solutions containing 60γ of copper, 1 ml. of the potassium nitrite solution, and 2 ml. of the $1M$ acetate buffer of pH 4.2. The total

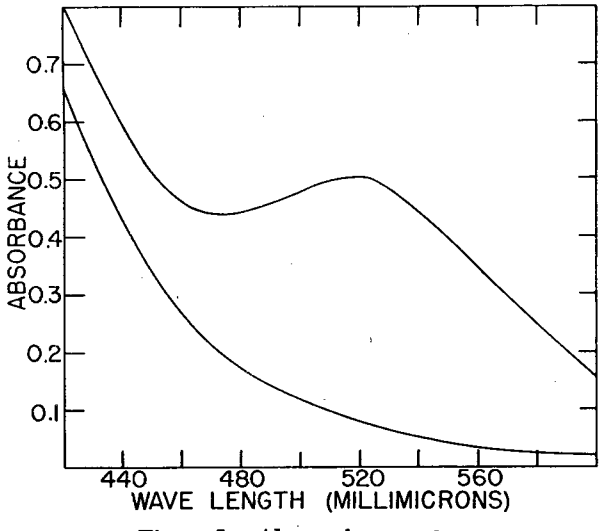

Figure 1. Absorption spectra

Upper curve, copper solutions
Lower curve, blanks

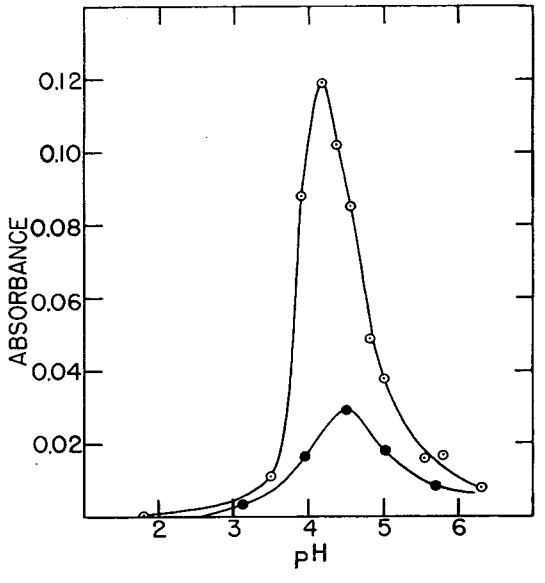

Figure 2. Effect of pH

Upper curve, copper solutions
Lower curve, blanks

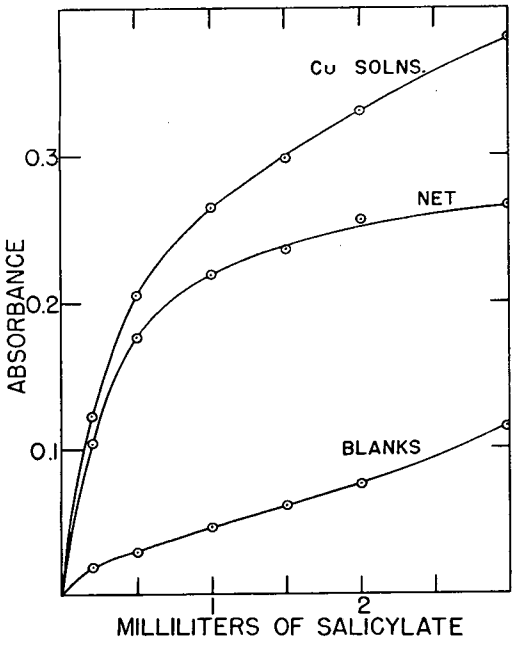

Figure 3. Effect of salicylate concentration

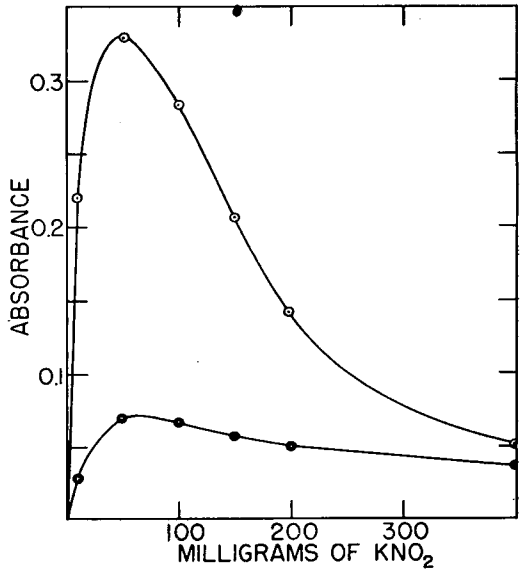

Figure 4. Effect of nitrite concentration

Upper curve, copper solutions
Lower curve, blanks

volume was 20 ml. in each case. A similar series of blanks was prepared, and the solutions were heated for 1 hour at 80° C. The results of this study are shown in Figure 3. Maximal color development requires about a twentyfold molar excess of salicylate with respect to copper.

Effect of Nitrite Concentration. Varying quantities of the potassium nitrite solution were added to a series of solutions containing 60γ of copper, 2 ml. of the $10^{-2}M$ sodium salicylate solution, and 2 ml. of the buffer. The total volume was 20 ml. in each case. A similar series of blanks was prepared, and the solutions were heated for 1 hour at 80° C. Figure 4 shows that the nitrite concentration has a marked effect on the color development, and that this effect is a complex one, with maximal color when about 50 mg. of potassium nitrite is present.

Effect of Duration of Heating. A series of solutions was prepared containing 60γ of copper, 2 ml. of the buffer, 2 ml. of the sodium salicylate solution, and 1 ml. of the potassium nitrite solution. The volume was 20 ml. A series of blanks was also prepared, and the solutions were placed together in a water bath at 80° C. At certain time intervals, solutions were removed from the bath and quickly cooled in ice water. The absorbances of the solutions are plotted in Figure 5, where it is seen that 1 hour is sufficient to ensure maximal color development. The practical difficulties in reproducing the heating and cooling make it necessary to run standards and unknowns through a determination together.

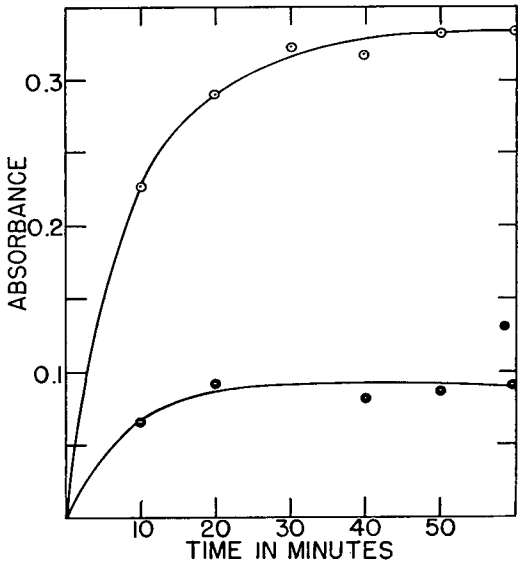

Figure 5. Effect of duration of heating

Upper curve, copper solutions
Lower curve, blanks

Stability of Colored Solutions. The red solutions, allowed to stand at room temperature, maintained constant absorbance values through an observation period of 2 hours.

ANALYTICAL RESULTS

A Beer's law plot obtained under optimal conditions as elaborated above was linear from 0 to about 8 p.p.m., after which a slight negative deviation appeared. A Ringbom plot of these same data showed that the minimal photometric error occurred when the copper concentration was about 5 p.p.m. Concentrations of about 2 and 9 p.p.m. embraced the range over which the method exhibited very nearly its maximal reproducibility.

Three groups of about 20 solutions each were prepared, containing 2.06, 5.15, and 10.3 p.p.m. of copper. The results obtained with these solutions are presented in Table I, where the range, median, mean, average deviation, and standard deviation are shown for each group. It seems clear from these results that the variables influencing the reaction are under reasonable control when conditions are regulated according to the above studies.

Interfering Metals. The changes in the absorbance values of solutions containing 5.15 p.p.m. of copper caused by the presence of 500 p.p.m. of several other metals were measured. Assuming the interference to be proportional to the concentration of interfering metal, the concentration of each metal was calculated which would produce an absorbance change of 0.0036 (1σ, Table I). These values also represent the concentrations of the metals which would produce the same absorbance value as 0.04 p.p.m. of copper. The tabulation (Table II) shows that the method is much more sensitive for copper than for the other metals tested. In addition to those metals in the table, iron(III), bismuth, and aluminum were tested. These three metals, as

expected, interfere by precipitating during the heating period at pH 4.2. Concentrations of bismuth and of aluminum which do not lead to precipitation do not interfere. Iron(III), on the other hand, leads to green rather than red solutions even when present to the extent of less than 1 p.p.m.

NATURE OF RED REACTION PRODUCT

It is necessary to heat solutions containing nitrite, salicylate, and cupric ion in order to obtain the red color within a reasonable period of time. It was observed, however, that the red color forms immediately at room temperature upon the addition of copper to a solution containing nitrite and salicylate which has been heated in the absence of copper. This eliminates from consideration such possibilities as a catalytic effect of cupric ion on some organic reaction between salicylate and nitrite. By breaking down the reaction into two stages, one involving nitrite and salicylate at elevated temperature and the other the reaction of copper with a product of the first reaction, insight has been gained into the mechanism of the color formation.

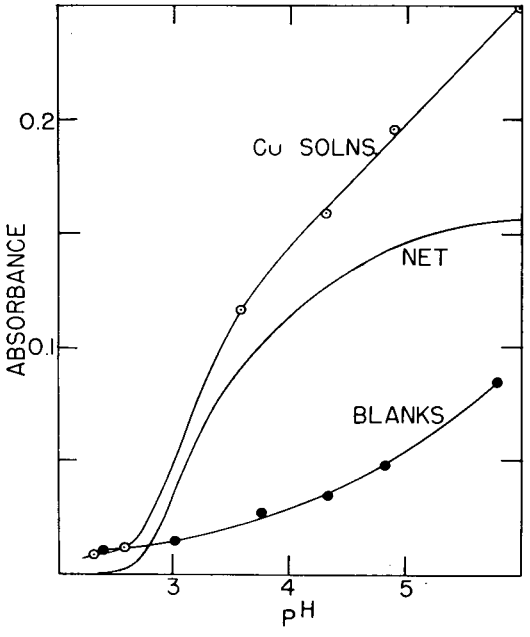

Figure 6. Effect of pH on isolated chelation step at room temperature

First, it was possible to show that once the reaction at elevated temperature is over, nitrite is no longer needed for color development. This was done simply by heating a solution containing nitrite and salicylate, then adding a large quantity of urea to the hot solution, followed by cooling it and adding the copper. The addition of urea, which is a well known method for destroying nitrites, did not obstruct the development of the red color. Thus it is certain that the red substance is not a nitrito complex of any sort; the possibility of reduction of cupric copper by nitrite is also eliminated.

Secondly, studying the affect of pH on the two separated phases of the reaction proved informative. A series of solutions containing nitrite and salicylate was buffered at various pH values and heated. After cooling to room temperature, all the solutions were adjusted to pH 6.0, and copper was added to each. The resulting curve of absorbance *vs.* pH was essentially the same as that for the over-all pH effect shown in Figure 2. A curve of this shape suggests the operation of opposing factors, which in the present case might be the effect of pH upon the ionization of salicylate acid and at the same time upon the stability of some necessary nitrous intermediate. On the other hand, a different curve is obtained for the effect of pH upon the reaction with copper at room temperature. A nitrite-salicylate solution, buffered at pH 4.2, was heated, then cooled, and aliquots were added to a series of copper solutions of various pH values. The final pH values, plotted against absorbance, are shown in Figure 6. The S-shaped curve thus obtained is typical of pH-dependent chelation reactions in general.

Table II. Interfering Metals

Metal Ion	Concn. Causing Error of 1σ in Determining 5.15 P.P.M. of Cu, P.P.M.
Pb	78
Ag	46
Cd	39
Hg(II)	13
Zn	10
Ni	2

It appears reasonable, then, to postulate that the red color in the Jorissen reaction is formed as follows: Nitrite and salicylate react at elevated temperature to form a chelating agent which can then react with copper to form a red product even at room temperature. The identity of this chelating agent has not been established. Authentic samples of 5-nitrososalicylic acid, 3-nitrosalicylic acid, and 5-nitrosalicylic acid do not give a red color with cupric ion. All of the observations which have been made during an attempt to find its nature are consistent with the interpretation that the free chelating agent is unstable, but that chelation with copper greatly stabilizes the molecule. The red solutions are stable for at least 2 hours. On the other hand, copper must be added almost immediately to the cooled nitrite-salicylate reaction mixture if the maximal quantity of red product is to be obtained. (This instability renders nonfeasible any improvement of the analytical method by preparing a solution of the chelating agent and later developing the color with copper at room temperature in calibrated glassware.)

The red material can be extracted into certain organic solvents, although it is sufficiently soluble in water that quantitative extraction cannot be made part of the analytical method. It is soluble in chloroform or ether, but does not extract into carbon tetrachloride or petroleum ether. When a red solution in chloroform is shaken with dilute acid (pH 1 to 2), the red color gives way to yellow in the organic phase, and copper appears in the aqueous layer. If no more than a few minutes intervene, the red color can be partially regenerated by shaking the yellow chloroform solution with aqueous copper at pH 6. However, the length of time required to process a large scale preparation (or even to measure the absorption spectrum with a manual instrument) is sufficient to destroy the chelating agent to the point where no red color with copper can be formed.

LITERATURE CITED

(1) Association of Official Agricultural Chemists, Washington, D. C., "Official Methods of Analysis of the Association of Official Agricultural Chemists," 7th ed., p. 469, 1950.
(2) Jorissen, *Bull. acad. roy. soc. Belg.* [3] **3**, 259 (1882).
(3) Müller, R. H., and Burtsell, A. T., *Mikrochemie ver. Mikrochim. Acta* **28**, 209 (1940).
(4) Schott, F., *Z. Nahr.-Genussm.* **22**, 727 (1911).
(5) Sherman, H. S., and Gross, A., *J. Ind. Eng. Chem.* **3**, 492 (1911).
(6) Snell, F. D., and Snell, C. T., "Colorimetric Methods of Analysis," 3rd ed., vol. 2, p. 136, Van Nostrand, New York, 1949.
(7) Welcher, F. J., "Organic Analytical Reagents," vol. 2, p. 123, Van Nostrand, New York, 1947.

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Colorimetric Determination of Moderate Concentrations of Uranium in Perchloric Acid Solutions

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In perchloric acid solution (2 to 65% by volume), at selected wave lengths, the colorimetric absorption of uranium solutions is proportional to the concentration. Uranium may be determined colorimetrically as uranium perchlorate, under these conditions. Above 65% perchloric acid (by volume) the absorbance values deviate from Beer's law, showing that a new species of uranium is present. These new species are postulated. Aluminum, cadmium, lead, iron, thorium, and zirconium perchlorate solutions do not absorb in the 415 to 420-mμ range.

THE photometric absorption of the soluble compounds of uranium has been the subject of extensive research in aqueous and nonaqueous solutions. Kaplan, Hildebrandt, and Ader (*8*) postulated formation of $[UO_2(NO_3)_3]^-$ in nitrate solution. Further work by the same authors (*7*) indicates that $(UO_2)^{++}$ and $[UO_2(NO_3)]^+$ may be found in nonaqueous solutions. Scott and Dixon (*11*) based their method on the absorption of uranyl salts in solution; maximum absorption is obtained in uranium solutions (limited amounts of chloride, fluoride, or nitrate) if the solution contains 10% sulfuric acid or 4% phosphoric acid (iron interferes).

Jones and Strong (*5*, *6*) and Müller (*9*) also reported work on absorption spectra for uranium solutions (acetate, fluoride, nitrate, sulfate).

Blake, Lowrie, and Brown (*2*) explained the importance of pH in their spectrophotometric investigation of the fluoride complexes. Sutton (*14*) also considered the importance of acidity; he worked in the acid range from about pH 0.2 to the point of hydrolytic separation.

Most of these investigations have been confined to determinations of small amounts of uranium, the best being those involving the use of thiocyanate (*3*, *4*, *12*), 8-quinolinol (*13*), and peroxide (*10*). The procedures for the latter two are concerned with amounts of uranium from fractional to milligram quantities, while the aqueous thiocyanate methods may use 1 to 3 mg. per ml.

The procedure described in this paper uses only higher amounts of uranium, 10 to 70 mg. per ml. The minimum volume of sample which may be used with the usual Beckman spectrophotometric setup is 4 ml., showing that this perchloric acid solution method is applicable only when higher concentrations of uranium perchlorate are to be determined.

The technique is brief. The sample (freed of sulfate) is evaporated to fumes with perchloric acid, cooled, and diluted with water–perchloric acid to a predetermined concentration of perchloric acid. After elimination of chlorine gas, the sample is cooled to 25° C. and read on the Beckman spectrophotometer.

In laboratory practice, the technique resembles Bastian's (*1*) differential procedure. The reference solution is a uranium solution, the concentration of which is slightly less than that of the unknown. A second standard solution of uranium which is slightly higher than that of the unknown is used to "bracket" the unknown.

An attractive feature of the method is the fact that those elements (which cause the greatest difficulties in other methods and require preliminary separations from uranium) do not interfere in the perchloric acid method.

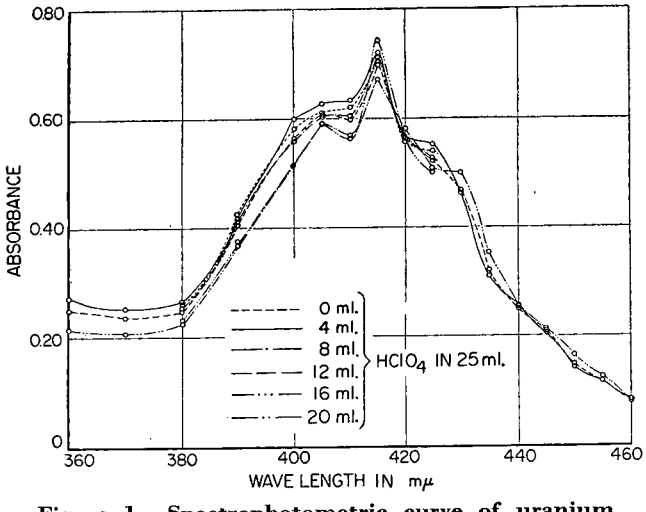

Figure 1. Spectrophotometric curve of uranium perchlorate in perchloric acid

0.595 gram of uranium in 25-ml. volume

SOLUTIONS AND APPARATUS

Uranium Perchlorate, 0.5*M*. Fume 71.52 grams of uranium trioxide with 55.3 ml. of perchloric acid for about 10 minutes. After cooling add 150 ml. of water, and boil the solution to expel free chlorine; filter off the white precipitate (silica). Dilute the filtrate to 500 ml. in a volumetric flask with water containing 1 to 100 perchloric acid, to prevent hydrolysis, and adjust to volume. One milliliter contains 118.8 mg. of uranium (gravimetric) (*13*).

Perchloric Acid, C.P., specific gravity 1.67, normality approximately 11.6.

Spectrophotometer. A Beckman Model DU spectrophotometer with 1-cm. silica cells is used for the colorimetric work.

PROCEDURE

To an unknown solution add 5 ml. of nitric acid and 15 ml. of perchloric acid. Evaporate down to heavy fumes of perchloric acid. Cool. Add water and heat on a steam bath for about 15 minutes to expel free chlorine. Transfer to a 25-ml. volumetric flask and dilute to volume with distilled water. Adjust the solution to 25° ± 0.3° C., and read the absorbance at selected wave lengths (415 to 420 mμ) with water as reference.

Optional Procedure. Determine the "approximate" concentration of the unknown uranium solution. Prepare two standard uranium solutions; one of which is slightly less and the other slightly higher than the "approximate" concentration of the unknown. Use the lower uranium standard as the reference solution. Calculate the unknown as the differential between the two standard solutions (*1*).

RESULTS

Six curves are shown in Figure 1, all of identical uranium content but of varying perchloric acid compositions. Coincidence of the curves is observed at wave lengths of 415 to 420 mμ, showing that uranium perchlorate solutions containing from 2 to 65% perchloric acid follow Beer's law, but the uranium solutions containing more than 65% of perchloric acid deviate from Beer's law and thus set the maximum acid content.

For six selected wave lengths, plots of absorbance against ura-

Table I. Effect of Diverse Elements on Determination of Uranium by Perchloric Acid

Sample	Uranium, Gram	Diverse Elements, Gram: Lead	Thorium	Zirconium	Absorbance at 417 mμ	Uranium Found, Gram
1	0.480	0	0	0	0.574	0.478
2	0.480	0.5	0	0	0.571	0.472
3	0.480	1.0	0	0	0.571	0.472
4	0.480	0	0.5	0	0.580	0.480
5	0.480	0	1.0	0	0.580	0.480
6	0.140	0	0	0	0.164	0.140
7	0.140	0	0	0	0.164	0.140
8	0.140	0	0	0.1	0.165	0.140
9	0.140	0	0	0.2	0.165	0.140

Table II. Determination of Uranium in Cadmium

Sample No.	Cadmium, Gram	Working Volume, Ml.[a]	Uranium, Gram: Expected	Found
1	1.0	25	0.3+	0.33
2	1.0	25	0.3+	0.33

[a] 60% perchloric acid by volume.

nium concentration are drawn and these straight lines are used as standard curves for the determination of uranium (Figure 2).

If a uranium standard solution is selected and the perchloric acid content is varied from 0 to 96% (by volume), an increase in absorbance is noted in the higher acid range (Figure 3). At 417 mμ and at an acidity greater than 70% perchloric acid (by volume), the increase in perchloric acid concentration causes an increase in absorbance. At 420 mμ and at an acidity greater than 65% perchloric acid (by volume), the increase in perchloric acid concentration causes an increase in absorbance. This increase in absorbance may be attributed to the formation of a new ionic species.

Aluminum perchlorate (0.1 gram), iron perchlorate (0.2 gram), thorium perchlorate (1.0 gram), and zirconium perchlorate (0.2 gram) do not absorb in the 415- to 420-mμ range (Figure 4).

Table I shows that the presence of 0.5 and 1.0 gram of lead, 0.5 and 1.0 gram of thorium, and 0.1 and 0.2 gram of zirconium has no effect on the determination of uranium. The spectrophotometric curve of zirconium perchlorate (Figure 4) presents further confirmation that at wave lengths of 415 to 420 mμ zirconium perchlorate does not absorb.

Data showing the results obtained when uranium is determined in a cadmium-uranium solution in the ratio of 3 to 1 are given in Table II. Reproducible results were obtained on duplicate samples. The entire 1.0 gram of cadmium sample was contained in a 25-ml. volume.

The uranium concentration of an unknown uranium perchlorate solution was determined by different methods. The results are shown in Table III, from which it is seen that excellent agreement was obtained from all the methods except those obtained by the gravimetric method. (The gravimetric results are sensitive to temperature variation and to the presence of small amounts of impurities.)

DISCUSSION

Analytical. From the analytical viewpoint, the determination of uranium in perchloric acid offers the following advantages:

Moderately large amounts of uranium may be determined with satisfactory precision. For example, the corresponding readings for 0.800 and 0.801 gram of uranium in 25 ml. of perchloric acid solution are 0.750 and 0.755, respectively, in a 10-mm. cuvette.

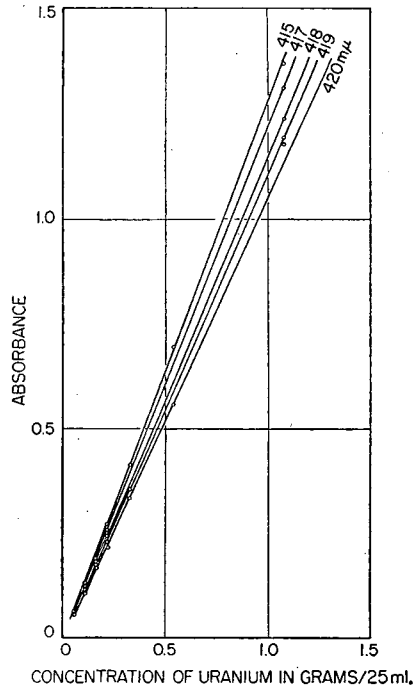

Figure 2. Absorbance curves of uranium perchlorate in perchloric acid

16 to 60% perchloric acid

Moderately large amounts of uranium may be determined without prior separation of iron or aluminum and the elements that most frequently accompany uranium. This is of advantage, as the separation of uranium from aluminum, iron, thorium, and zirconium (and other elements which hydrolyze below pH 6) requires special handling when uranium is to be determined gravimetrically. It is applicable for the direct determinations of $UO_3 . xH_2O$ (yellow) and of UO_2^{++} (black U_3O_8) which are directly soluble in a nitric-perchloric acid mixture.

Moderately large amounts of uranium may be separated from silicon, niobium, tantalum, tungsten, and tin (small amounts) by simple filtration of the perchloric acid solution, and the filtrate is ready for absorption measurements.

Interfering complexing agents such as fluoride, chloride, and carbonate are removed during the perchloric acid fuming stage.

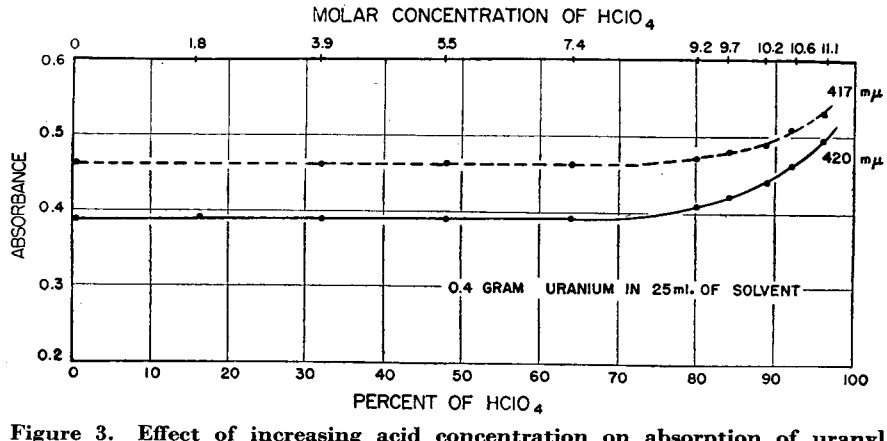

Figure 3. Effect of increasing acid concentration on absorption of uranyl perchlorate

Table III. Standardization of a Uranium Solution

(Comparison of methods)

Sample No.	Uranium, Mg. per Ml. Colorimetric	Potentiometric	Volumetric	Gravimetric	Gravimetric colorimetric[a]
1a	14.0	14.0	14.0	13.8	14.0
1b	14.0	13.9	14.0	13.8	14.0
1c	14.2	13.9	14.0	13.7	13.8
1d	13.9		14.0	13.9	13.9
2a	14.0		14.0	13.77	
2b	14.0		14.0	13.77	
2c	14.2		14.0	13.8	
2d	14.2		14.0	13.9	
				13.73	
				13.74	

[a] Uranium determined first by gravimetric method, followed by colorimetric analysis of precipitate.

(Sulfuric acid from uranium sulfate is evolved at 350° C. with ammonium carbonate.)

Ionic Species in Uranyl Solutions. The absorbance of uranyl perchlorate solutions increases rapidly and deviates from Beer's law (Figure 3) as the concentration of the perchloric acid medium increases beyond the 65 to 80% range.

The deviation may be attributed to complex formation of the following types:

$$UO_2(ClO_4)_2 + HClO_4 \rightarrow HUO_2(ClO_4)_3 \quad (1)$$

$$UO_2(ClO_4)_2 + mHClO_4 \rightarrow H_mUO_2(ClO_4)_{m+2} \quad (2)$$

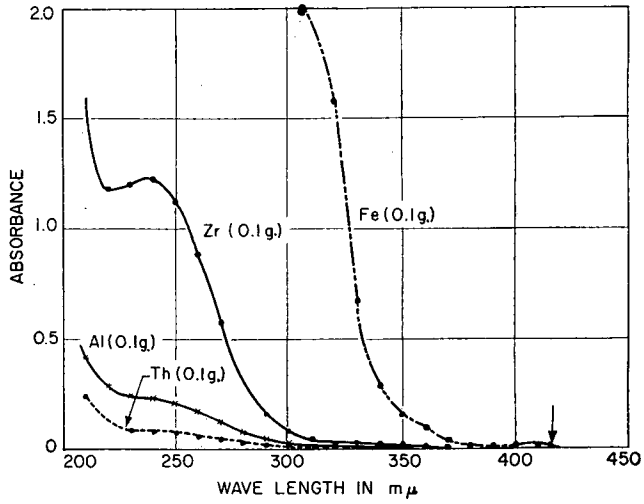

Figure 4. Spectrophotometric curves for certain perchlorates (40% perchloric acid)

× 100 mg. of aluminum in 25 ml.
—·— 100 mg. of iron in 25 ml.
---- 100 mg. of thorium in 25 ml.
—— 100 mg. of zirconium in 25 ml.

LITERATURE CITED

(1) Bastian, R., ANAL. CHEM. 21, 972 (1949).
(2) Blake, C. A., Lowrie, R. S., and Brown, K. B., Oak Ridge National Laboratory, Oak Ridge, Tenn., "Spectrophotometric Investigation of the Uranyl Fluoride Complexes," Rept. AECD-3212 (May 24, 1951).
(3) Currah, J. E., and Beamish, F. E., ANAL. CHEM. 19, 609 (1947).
(4) Gerhold, M., and Hecht, F., *Mikrochemie ver. Mikrochim. Acta* 36, 1100 (1951).
(5) Jones, H. C., and Strong, W. W., "Absorption Spectra of Solutions," Carnegie Inst., Wash., Pub. 160 (1911).
(6) Jones, H. C., and Strong, W. W., "Study of Absorption Spectra," *Ibid.*, 130 (1910).
(7) Kaplan, L., Hildebrandt, R. A., and Ader, M., Argonne National Laboratory, Lemont, Ill., "Absorption Spectra of Uranyl Nitrate in Organic Solvents," Rept. ANL-4521 (Oct. 18, 1950).
(8) Kaplan, L., Hildebrandt, R. A., and Ader, M., Argonne National Laboratory, Lemont, Ill., "Trinitratouranyl Complex in Ketonic Solvents," Rept. ANL-4520 (Oct. 18, 1950).
(9) Müller, A., *Ber.* 73B, 1353–8 (1940).
(10) Rodden, C. J., "Analytical Chemistry of the Manhattan Project," McGraw-Hill, New York, 1950.
(11) Scott, T. R., and Dixon, P., *Analyst* 70, 462 (1945).
(12) Silverman, L., and Moudy, L., North American Aviation, Inc., Downey, Calif., "Photometric Determination of Uranium as Thiocyanate–Penta Ether Extraction Method," Rept. NAA-SR-244 (June 17, 1953).
(13) Silverman, L., Moudy, L., and Hawley, D., ANAL. CHEM. 25, 1369 (1953).
(14) Sutton, J., Chalk River Laboratory, Canada, "Ionic Species in Uranyl Solutions," Rept. CRC325 (March 21, 1947).

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Investigation of Color Reaction between *p*-Dimethylaminobenzaldehyde and Urea or Ureido Acids

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Studies of the color test employed for β-ureido acids suggest that it involves an equilibrium reaction between $RNHCONH_2$ and the hydrochloride of *p*-dimethylaminobenzaldehyde, with formation of a Shiff base hydrochloride. Color development was found to be strongly suppressed by dilution, as expected for a reversible reaction of this type, and it was also decreased by hydrochloric acid concentrations either above or below the optimal level. Together, these findings offer an explanation of the complex effects of environmental factors noted when the color reaction was carried out on filter paper, provide a more rational basis for setting up controls in routine chromatographic analyses, and yield some indirect information concerning the nature of conditions existing in or on sprayed filter paper.

FOR β-ureido acids and related compounds *p*-dimethylaminobenzaldehyde (PDAB) has proved to be a sensitive color reagent on paper chromatograms (*2*). Quantitative studies, however, rather frequently showed unanticipated variations in the rate and intensity of color development in chromatographic spots and surrounding background areas, indicating the need for more basic investigations of factors involved in the color reaction.

APPARATUS

A modified Perkin-Elmer flame photometer and a Beckman Model DU spectrophotometer were used as described previously (*2*) for studying the color reaction on filter paper, and the latter instrument was used for measurements in solution.

COLOR REACTION ON PAPER

Reagent Variations. In order to test the effect of varying the

sodium hydroxide and hydrochloric acid concentrations in the spray reagents, filter paper sheets were dipped into a dihydrouracil (DHU) solution, dried, sprayed with various sodium hydroxide solutions as separate vertical stripes, dried again, and sprayed in separate horizontal stripes with *p*-dimethylaminobenzaldehyde solutions containing various concentrations of hydrochloric acid. The areas where stripes intersected were then measured photometrically at intervals, along with similarly sprayed sheets containing no dihydrouracil.

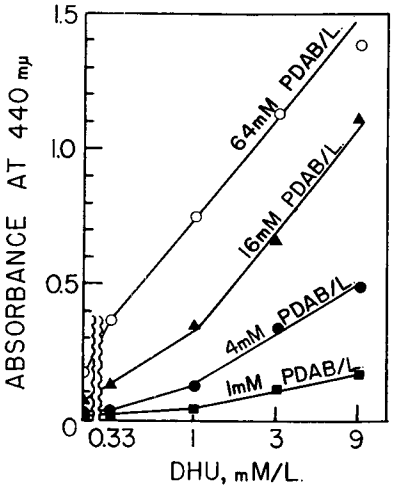


Figure 1. Concentration effects in cross-sprayed filter paper

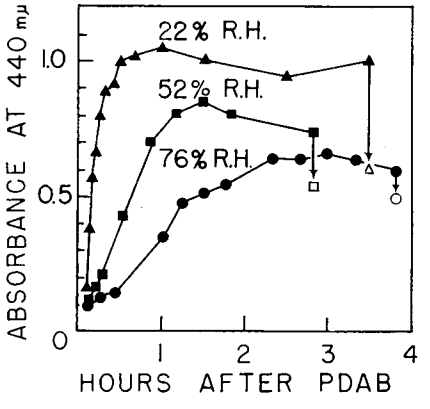


Figure 2. Effect of relative humidity on color development in paper

Solid points, total color
Open points, minus background

Recorded in Table I, the results are set up in a form resembling the spraying pattern employed. The data indicated that the reagent background color was increased by long standing and by high concentrations of chlorides in the paper, that color development was prevented by failure of the final spray to acidify the paper, but only slightly reduced and delayed by excessive acidification, and that the hydrolysis of dihydrouracil was incomplete with alkali concentrations of 0.25*N* or less. Previous data had indicated that hydrolysis of dihydropyrimidines was essentially complete after treatment with 0.5*N* sodium hydroxide (*2*), and use of higher concentrations has not appeared to be advantageous.

The effect of *p*-dimethylaminobenzaldehyde concentration on

Table I. Effect of Varying Sodium Hydroxide and Hydrochloric Acid Concentrations in Spray Reagents[a]

	0.063*N* NaOH			0.125*N* NaOH			0.25*N* NaOH			0.5*N* NaOH		
0.25*N* HCl	(0) 6	(2) 12	(5) 12	(1) 16	(5) 28	(7) 27	(1) 44	(4) 64	(8) 54	(0) 2	(3) 0	(3) 0
0.5*N* HCl	(0) 5	(4) 10	(6) 11	(1) 10	(4) 26	(8) 29	(1) 26	(3) 68	(7) 64	(2) 60	(5) 96	(9) 77
1.0*N* HCl	(1) 2	(5) 7	(5) 11	(1) 4	(5) 24	(6) 28	(1) 9	(3) 58	(7) 55	(2) 20	(5) 89	(11) 72
2.0*N* HCl	(1) 0	(3) 9	(8) 12	(0) 2	(4) 22	(7) 26	(0) 6	(5) 57	(14) 57	(2) 8	(11) 85	(21) 72

[a] Each group of three figures in parentheses shows the reagent background color (as $A_{440} \times 100$) developed in 0.5, 6.5, and 19 hours after spraying with alkali of normality tabulated above, and with 0.06*N* *p*-dimethylaminobenzaldehyde in hydrochloric acid of the normality indicated at the left. Each group of three figures without parentheses similarly show the additional color developed after a preliminary dip of the paper in 0.003*N* dihydrouracil.

color development and background intensity was similarly investigated by first spraying duplicate filter paper sheets in separate vertical stripes with dihydrouracil of different normalities, drying, spraying uniformly with 0.5*N* sodium hydroxide, drying again, and spraying separate horizontal stripes of ethanolic 1*N* hydrochloric acid containing different concentrations of *p*-dimethylaminobenzaldehyde. Absorbance measurements made 3 hours later (Figure 1) indicated that the sensitivity of the color reaction was lowered by dilution of the color reagent used in routine chromatography, even when the reduced concentration still greatly exceeded that of the dihydropyrimidine. Conversely, increasing the concentration of the routine reagent could provide a further increase in sensitivity, but this appeared to cause more frequent and severe difficulties from intense background color and was not studied systematically.

Brief tests of other reagent variations suggested that lower background color and/or greater sensitivity were obtained by recrystallizing the commercial *p*-dimethylaminobenzaldehyde, by making it up as a fresh solution once a week and storing it in the refrigerator, and by using ethyl alcohol–water mixtures for making up both of the spray reagents. Addition of a small amount of sulfuric acid to the final spray showed no marked effect on color development or fading.

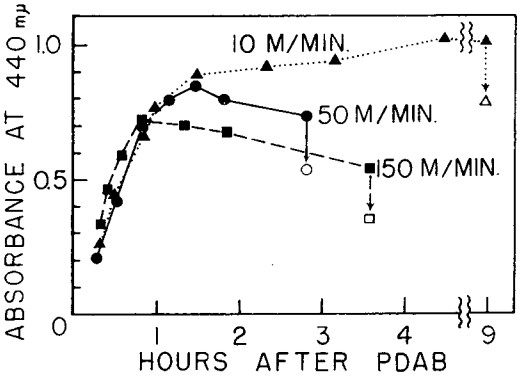


Figure 3. Effect of rate of air flow on color development in paper

Solid points, total color
Open points, minus background

Environmental Variations. Observations during routine dihydropyrimidine analyses gave the impression that changes in relative humidity might be influencing the color reaction on paper. In confirmation it was found that at very high humidity,

with freshly sprayed paper strips suspended in closed jars over water, dilute sodium hydroxide, or saturated potassium bromide (84% relative humidity), neither a dihydrouracil spot nor a background color appeared. At very low humidity in jars containing phosphoric anhydride or solid potassium hydroxide, on the other hand, a rapidly developing intense background color completely obscured the dihydrouracil spot.

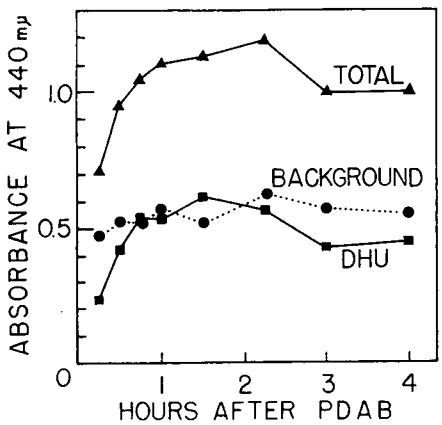


Figure 4. Color development in paper at 22° C.

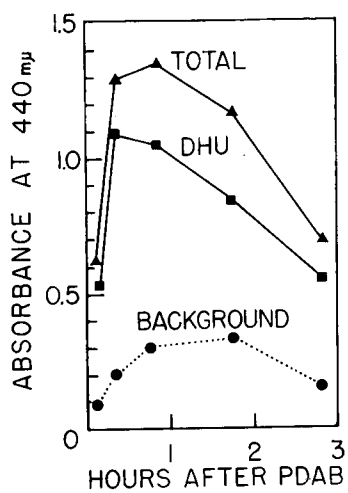


Figure 5. Color development in paper at 33° C.

Intermediate humidities yielded equivocal results in closed systems and so were further investigated by placing sprayed paper strips in a glass tube clamped to the spectrophotometer and drawing a stream of conditioned air through the tube. At a constant temperature (26° C.) and air flow (50 meters per minute), lowering the humidity accelerated and intensified over-all color development (dihydrouracil spot plus background), with the background intensity accounting for a major portion of the differences existing 3 to 4 hours after spraying (Figure 2). Background measurements are represented by the length of the vertical arrow at the end of each curve, and were omitted at earlier time intervals in order to avoid disturbing the orientation of the paper in the light beam. In obtaining the data for Figure 2 the light was turned on very briefly for each reading and no local effects on the color reaction were apparent, but prolonged exposure to the strong light occasionally caused the area under the beam to become lighter or darker than the surrounding areas, or even to develop a blue color similar to that shown by some initially yellow chromatographic spots after long storage. With the same technique it was found (Figure 3) that at 26° C. and 52% relative humidity, increasing the rate of air flow past the paper decreased both the peak color intensity and the time required to reach peak intensity. In this case the background color contributed little to the final differences.

Development of the dihydrouracil and background colors were followed individually in an air-conditioned room where the papers were kept in front of a fan, except during spectrophotometer readings. With a relative humidity of 51% and an air flow of about 150 meters per minute, raising the temperature from 22° to 33° C. increased the peak intensity of the dihydrouracil spot and its rate of development but lowered the background absorbance (Figures 4 and 5). This effect on background may have been mediated through the higher absolute humidity at the elevated temperature, but the other effects seem more complex.

COLOR REACTION IN SOLUTION

Interpretations based only on filter paper data such as those outlined above were made difficult by the lack of detailed information concerning the characteristics of the "solution" remaining in or on the cellulose fibers at any given time after spraying, and study of the color reaction was continued with solutions of known composition. Absorption spectra are shown in Figure 6, curves *A* through *E*, for yellow solutions obtained by addition of *p*-dimethylaminobenzaldehyde and hydrochloric acid to aqueous solutions of various compounds of the general formula $RNHCONH_2$, in amounts such that final concentrations of the three solutes were 0.02*N*, 0.2*N*, and 0.02*N*, respectively. Although the different compounds gave similar absorption curves, the intensity of the color reaction was affected by the nature of the R group, with the β-ureido acids being the most chromogenic. Many of the colored acidic solutions appeared to be

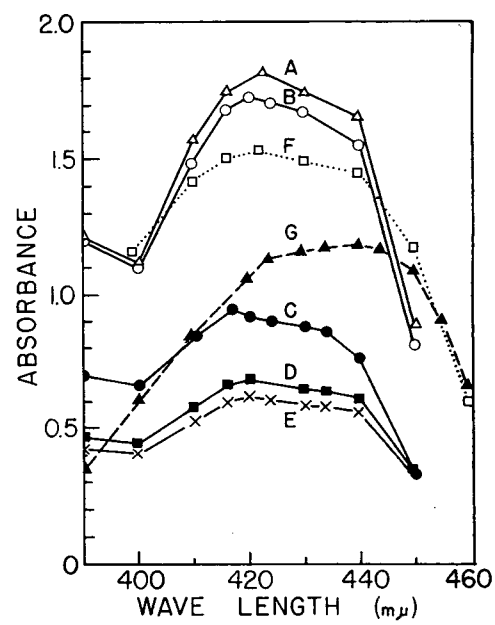


Figure 6. Absorption spectra developed in solution (*A* to *E*) and in paper (*F*, *G*)

A. β-Ureidoisobutyric acid
B. β-Ureidopropionic acid
C. Urea
D. α-Ureidopropionic acid
E. Ureidosuccinic acid
F. Urea
G β-Ureidoisobutyric acid

stable indefinitely, but those containing a β-ureido acid were sometimes partially decolorized over a period of several days, possibly because of cyclization, and some containing relatively inactive compounds such as succinylurea developed color slowly, presumably through hydrolytic release of urea. Filter paper spots showing absorption curves similar to those of the above solutions could be produced by minimizing the background color through use of low *p*-dimethylaminobenzaldehyde concentrations. For example, Figure 6, *F* was obtained by drying on filter paper a 4-μl. droplet of aqueous 0.4*N* urea, dipping it in a solution containing *p*-dimethylaminobenzaldehyde (0.01*N*) and sulfuric acid (0.05*N*), and mounting the spot in the spectrophotometer curvette holder along with an adjacent sprayed area of the filter paper which served as the blank. The routine chromatographic color development procedure, on the other hand, generally yielded an absorption curve similar to Figure 6, *G*, which was derived from a 4-μl. droplet of 0.01*N* β-ureidoisobutyric acid treated with the usual spray reagents. Here the absorption peak was shifted to a longer wave length, suggesting that the curve was overcorrected for the reagent background absorption, which increases rapidly toward the shorter wave lengths, with a maximum at 353 mμ.

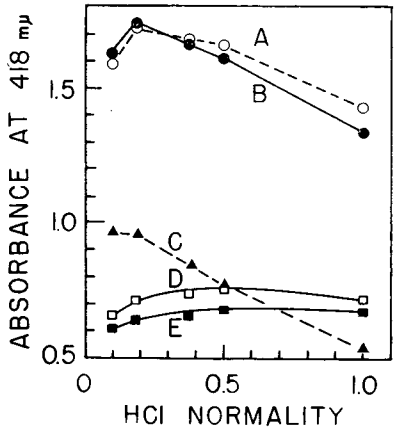


Figure 7. Effect of hydrochloric acid concentration on color reaction in solution

A. β-Ureidoisobutyric acid
B. β-Ureidopropionic acid
C. Urea
D. α-Ureidopropionic acid
E. Ureidosuccinic acid

As the room temperature was increased, the absorbance decreased by about 2% per degree in solutions containing moderate concentrations of hydrochloric acid, *p*-dimethylaminobenzaldehyde, and urea or a derivative—e.g. 0.2*N*, 0.02*N*, and 0.02*N*, respectively. With a high concentration of either *p*-dimethylaminobenzaldehyde or urea, however, the effect was reversed to yield a positive temperature coefficient (2% per degree for 0.2*N* hydrochloric acid, 0.0002*N* *p*-dimethylaminobenzaldehyde, 4*N* urea; 4% per degree for 6*N* hydrochloric acid, 2*N* *p*-dimethylaminobenzaldehyde, 0.0005*N* urea) which may account, at least in part, for the intensified color reaction on filter paper at elevated temperatures.

In solution, as on paper, either too much or too little hydrochloric acid prevented maximum color development, and the optimum acidity was found to differ for solutions containing various types of reactants, as shown in Figure 7 for solutions 0.02*M* in reactant and in *p*-dimethylaminobenzaldehyde.

At room temperature and with the initial hydrochloric acid concentration set at 0.2*N*, the absorbance of aqueous solutions tended to be proportional to the product of the initial concentrations of reagent and reactant unless one of these was present in overwhelming excess (Figure 8), indicating that formation of the colored product involves a reversible equilibrium reaction. The marked effect of dilution on such reactions could presumably account for the low sensitivity of the color reaction in dilute solutions or on damp filter paper as compared with its very high sensitivity on nearly dry filter paper. The difference is clearly indicated in Figure 6, where curve *A* represents an aqueous solution containing a total amount of β-ureidoisobutyric acid at least 1500 times that present in the filter paper spot used for obtaining curve *G*.

Taken together, the acidity and dilution effects offer at least a qualitative explanation for most of the complex effects of humidity, air flow, and temperature on the chromatographic color reaction, because these factors would affect the rate of loss of hydrochloric acid from the sprayed paper and the re-establishment of moisture equilibrium between the paper and the atmosphere. Either process could constitute the limiting factor in the rate of color development, and the maximum absorbance attained by a given spot might be expected to vary approximately as the inverse square of the moisture content of the paper at the time when the hydrochloric acid concentration was optimal. Continuing loss of acid through evaporation, or through neutralization by atmospheric contaminants, probably accounts for most of the subsequent fading of colored spots, but fading could also result from a further gain of water or from loss of other components of the system through volatilization or side reactions. The ethyl alcohol in the final spray reagent probably evaporates long before the time of maximal color development and thus may function primarily in limiting the amount of water initially applied to the paper, though the color reaction in solutions can also be intensified to a moderate degree by using aqueous ethyl alcohol (or aqueous sodium chloride) in place of pure water as the solvent.

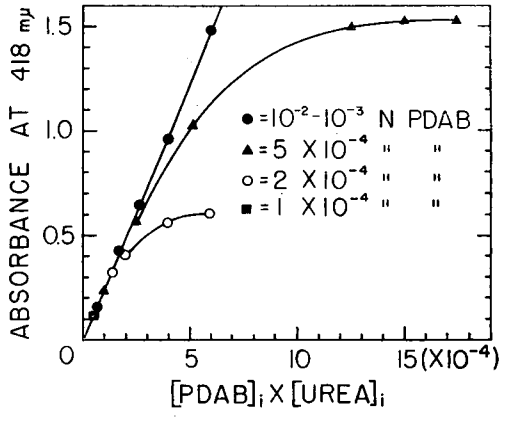


Figure 8. Color development in solutions *vs.* product of initial concentrations of *p*-dimethylaminobenzaldehyde and urea

A high "blank" absorption similar to that noted on very dry filter papers was sometimes encountered when the color reaction in aqueous solutions was forced toward completion by evaporating the water and extracting the dry residue with chloroform. The high blank, or background, color has not been extensively studied, but may represent a side reaction or a hypersensitization to impurities in vigorously dehydrated systems containing *p*-dimethylaminobenzaldehyde and hydrochloric acid.

The dehydration-extraction technique and other procedures employing nonaqueous solvents can greatly increase the sensi-

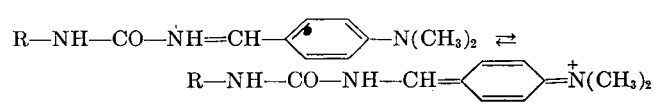
tivity of the color test but appear difficult to adapt for quantitative uses such as spectrophotometric measurements of eluted chromatographic spots. Chloroform does not extract the yellow color from filter paper spots or "other" aqueous solutions, while elution (and dilution) with water destroys the color. Dehydration of the eluate can restore the color and render it extractable by chloroform, but the chloroform solution is more or less rapidly decolorized on standing (with a simultaneous increase in the characteristic ultraviolet absorption of free *p*-dimethylaminobenzaldehyde), thus tending to make such an elution procedure inaccurate, as well as inconvenient.

Use of the *p*-dimethylaminobenzaldehyde reagent for studying the hydrolysis of dihydropyrimidines in solution showed them to be even more sensitive to alkali than had been suspected from the studies with filter paper. Adding an equimolar amount of sodium hydroxide to 0.01*N* dihydrouracil at room temperature gave over 50% hydrolysis in 6 hours, whereas adding a 2.5-fold excess of alkali caused essentially complete hydrolysis within 3 hours.

MECHANISM STUDIES

Extending the spectrophotometric measurements into the ultraviolet yielded what appears to be an over-all picture of the reactions leading to color development. Increasing the acidity of an aqueous *p*-dimethylaminobenzaldehyde solution (Figure 9, curves *A* and *B*) reduced the absorbance (*A*) at 353 mμ and increased it at 242 mμ, apparently representing a conversion of the amine to its cationic form in accordance with related data of Kumler and Strait (*3*). These two curves permit estimation of the molar absorptivity of the cationic and the unchanged forms as ϵ_{242} equals 14,000 and ϵ_{353} equals 46,000, respectively, using the assumption that for each curve the concentration of the hydrochloride (taken as A_{242}/ϵ_{242}) plus that of the free base (taken as A_{353}/ϵ_{353}) equals the concentration of the amine as initially added to the mixture. These values yield an equilibrium constant of about 140 for a simple ionization reaction of the form PDAB + H^+ ⇄ PDAB·H^+. Comparison of curves *B* and *C* of Figure 9 then suggests that the molarity of the colored product (taken as A_{420}/ϵ_{420}) plus the apparent slight increase in unionized *p*-dimethylaminobenzaldehyde, may be equated to the apparent decrease in the cationic form of the reagent. Such calculations yield a molar absorptivity (ϵ_{420}) of about 6000 for the colored product, and an equilibrium constant of about 0.6 for a reversible reaction of the form: PDAB·H^+ + $RNHCONH_2$ ⇆ colored product. These two equilibria probably represent the major reactions involved in the color development, but additional complex effects apparently occur, for the "constants" tend to drift with changing experimental conditions. For example, gradually increasing the acid concentration of a *p*-dimethylaminobenzaldehyde solution not only eliminated absorption at 353 mμ, but eventually began also to suppress the 242-mμ peak and reduce the solution's capacity for producing color with $RNHCONH_2$. Increasing the urea concentration similarly produced smaller and smaller increments in A_{420} per unit decrease in A_{242}, so that for urea concentrations of 0.5, 1, 2, 4, and 6*M*, the calculated values for $\epsilon_{420} \times 10^{-3}$ were approximately 11, 9, 6, 4.5, and 4, respectively. The molar absorptivities calculated in this manner were not markedly affected by three- to fivefold changes in *p*-dimethylaminobenzaldehyde or hydrochloric acid concentrations, but substitution of 0.5*M* β-ureidoisobutyric acid for 0.5*M* urea nearly doubled the value for ϵ_{420} and also increased the calculated equilibrium constant from 0.27 to 0.34, indicating that the nature of the R group in $RNHCONH_2$ may influence both the yield and the optical properties of the colored product.

The easy reversibility of the color reaction and the light-absorbing properties of the solutions suggested that the yellow products might be simple Schiff bases with quinoid resonating structures such as the following:



Products isolated after addition of *p*-dimethylaminobenzaldehyde and hydrochloric acid to concentrated solutions of β-ureidopropionic acid or urea yielded analyses compatible with such Schiff base hydrochloride structures, but showed a lower stability than anticipated from data reported for dimethylaminobenzalurea as the free base (*1*). When *p*-dimethylaminobenzaldehyde and β-ureidopropionic acid (0.01 mole of each) were dissolved in 20 ml. of ethyl alcohol, 5.5 ml. of water and 1 ml. of concentrated hydrochloric acid, a fluffy yellow precipitate formed on standing at 0° C. This product, after drying in vacuum over sodium hydroxide for 1 hour, weighed 0.44 gram (15% of theoretical) and melted at 162–164° C.

$C_{13}H_{18}O_3N_3Cl$ (299.8).	Calculated.	C 52.08, H 6.05, N 14.02, Cl 11.83
	Found.	C 51.29, H 6.07, N 13.90, Cl 11.12

The product showed a diphasic titration curve and a neutral equivalent of 153 (calculated: 150). Extraction of the neutralized solution with ethyl ether resulted in recovery of 93% of the theoretical yield of pure *p*-dimethylaminobenzaldehyde, and β-ureidopropionic acid could be detected chromatographically in the aqueous residue. On making up a 0.6% (theoretically 0.02*N*) solution of the product in aqueous 0.2*N* hydrochloric acid, the initially intense yellow color faded rapidly to a stable absorbance of 1.76 at 420 mμ, whereas an aqueous solution of 0.02*N* β-ureidopropionic acid, 0.02*N* *p*-dimethylaminobenzaldehyde, and 0.2*N* hydrochloric acid apparently approached the same equilibrium point from the opposite direction, rapidly increasing in absorbance to a value of 1.74 at 420 mμ. A 200-fold dilution of the 0.02*N* solution of the product with 0.1*N* hydrochloric acid apparently shifted the equilibrium almost entirely in the direction of dissociation, leaving no significant absorption at 420 mμ, but giving absorbance readings at 242 and 353 mμ of 1.20 and 0.51, respectively, very close to the comparable readings of 1.24 and 0.52 found for a $10^{-4}N$ solution of pure *p*-dimethylaminobenzaldehyde in 0.1*N* hydrochloric acid.

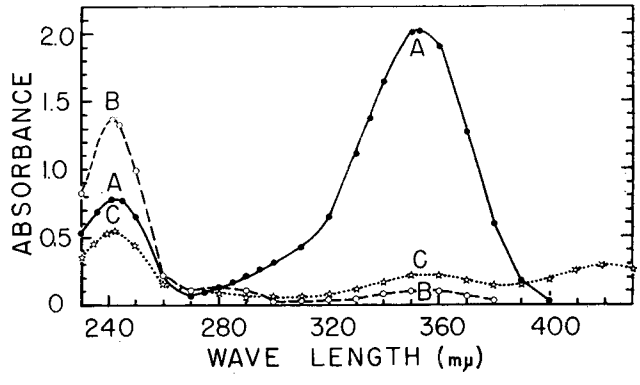


Figure 9. Absorption spectra of aqueous $10^{-4}N$ *p*-dimethylaminobenzaldehyde with

A. 0.01*N* HCl
B. 0.4*N* HCl
C. 0.4*N* HCl, 2*N* urea

To test the possibility that 2 moles of reagent might combine with 1 mole of urea, a mixture consisting of 0.05 mole of *p*-dimethylaminobenzaldehyde, 0.025 mole of urea, 60 ml. of ethyl alcohol, 20 ml. of water, and 6 ml. of hydrochloric acid was refrigerated, and the resulting precipitate was filtered and shielded from light while drying in vacuo over sulfuric acid for several weeks. The product weighed 3 grams, decomposed at 230–232°

C., and its elementary analysis approximated that calculated for the reaction of equimolar amounts of urea and *p*-dimethylaminobenzaldehyde hydrochloride:

$C_{10}H_{14}ON_3Cl$ (227.7). Calculated. C 52.75, H 6.20, N 18.46, Cl 15.57
Found. C 51.30, H 6.19, N 17.97, Cl 16.15

DISCUSSION

The experiments outlined have indicated that it would be difficult to determine and maintain a set of conditions giving optimum sensitivity and reproducibility in routine use of *p*-dimethylaminobenzaldehyde for chromatographic estimation of pyrimidine reduction products, though setting up such conditions might be justified if a large number of analyses were required. Fortunately, the sensitivity of the color reaction is within a usable range under most of the conditions of temperature and humidity ordinarily encountered in the laboratory, and the factor of air flow may be standardized, to a large extent, by adoption of a reasonably consistent procedure for spraying and drying the chromatograms. Comparison of chromatograms of an unknown sample with simultaneously prepared chromatograms of standard solutions then tends to correct for day to day variations in sensitivity, and by increasing the number of chromatograms used the accuracy of a given estimation can usually be brought within the required limits without an inordinate expenditure of time and effort.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Bellavita, V., and Cagnoli, N., *Gazz. chim. ital.* **69**, 583, 602 (1939).
(2) Fink, R. M., Cline, R. E., McGaughey, C., and Fink, K., ANAL. CHEM. **28**, 4 (1956).
(3) Kumler, W. D., and Strait, L. A., *J. Am. Chem. Soc.* **65**, 3492 (1943).

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Fractionation of Hydrocarbons by Azeotropic Distillation with Fluorochemicals

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Certain fluorochemical compounds were discovered to form, with the several classes of hydrocarbons, a series of azeotropic mixtures whose boiling points are in an order just the reverse of that for the usual polar azeotrope-forming substances, such as alcohols, glycol ethers, etc. For the binary azeotropic mixtures formed between a fluorochemical and a paraffin and cycloparaffin hydrocarbon, respectively, both having about the same normal boiling point, the boiling point is lower for the cycloparaffin azeotrope and higher for the paraffin azeotrope. The complete resolution of a mixture of branched paraffins and cycloparaffins into two portions, one portion containing only branched paraffins, the other only cycloparaffins, can be effected by azeotropic distillation using fluorochemicals alternatively with polar organic compounds as azeotrope-forming substances.

THE American Petroleum Institute Research Project 6 has for many years relied very heavily on the process of azeotropic distillation for the separation of various types of hydrocarbons. Heretofore in this work, polar organic compounds containing hydroxyl, ether, acid, cyanide, or similar groups have been used as azeotrope-forming substances (*3*, *4*). Certain fluorochemicals form, with the several classes of hydrocarbons, a series of azeotropic mixtures whose boiling points are in an order just the reverse of that for the usual polar azeotrope-forming substances, such as alcohols and glycol ethers. This paper describes the results obtained with this new class of azeotrope-forming substances, the fluorochemicals.

For the binary azeotropic mixtures formed between a polar organic compound containing hydroxyl, keto, cyanide, or similar groups, and a paraffin, a cycloparaffin, and an aromatic hydrocarbon, respectively, all having about the same normal boiling point, the boiling point of the binary azeotropic mixture is lowest for the paraffin azeotrope, intermediate for the cycloparaffin azeotrope, and highest for the aromatic azeotrope. On the other hand, for the binary azeotropic mixtures formed between paraffin and cycloparaffin hydrocarbons, respectively, and certain fluorochemicals, the reverse is the case with the cycloparaffin azeotrope having a lower boiling point than the paraffin azeotrope.

EXPERIMENTAL RESULTS

Three mixtures were tested in this investigation.

Equal volumes (about 175 ml. each) of 2,2,4-trimethylpentane and methylcyclohexane, with the perfluorocyclic ether, $C_8F_{16}O$ (Minnesota Mining and Manufacturing Co., No. 0-75. Believed to consist of a fluorinated side chain attached to a ring of 5 or 6 atoms, including oxygen.)

Nearly equal volumes (25 and 29 ml., respectively) of 2,3,5-trimethylhexane and ethylcyclohexane, with the perfluorocyclic ether, $C_8F_{16}O$.

Nearly equal volumes (90 and 100 ml., respectively) of 3,3,5-trimethylheptane and *n*-propylcyclohexane, with heptacosa-

Table I. Data on Azeotropic Mixtures Involving Hydrocarbons and Fluorochemicals

Hydrocarbon	Azeotrope-forming Substance	Boiling Point, ° C. at 760 Mm. Hg: Hydrocarbon	Azeotropic mixture	Azeotrope forming substance	Approx. Amt. of Hydrocarbon in Azeotropic Mixture, % Vol.
2,2,4-Trimethylpentane	Perfluorocyclic ether, $C_8F_{16}O$	99.24	87.5	102.5	40
Methylcyclohexane		100.93	85.0	102.5	40
2,3,4-Trimethylhexane		131.34	98.4	102.5	20
Ethylcyclohexane		131.78	96.3	102.5	20
3,3,5-Trimethylheptane	Heptacosafluorotributylamine, $(C_4F_9)_3N$	155.68	147.3	178.4	55
n-Propylcyclohexane		156.72	145.4	178.4	55

fluorotributylamine, $(C_4F_9)_3N$ (Minnesota Mining and Manufacturing Co., No. N-43).

The hydrocarbon material was recovered from the azeotropic distillate by extraction with ethyl alcohol at low temperatures, using one-half volume of ethyl alcohol, and subsequently washing the alcoholic extract with water to separate the hydrocarbon. Where the perfluorocyclic ether, $C_8F_{16}O$, was used, the azeotropic distillate was extracted at −80° C. With heptacosafluorotributylamine, the extraction with ethyl alcohol was conducted at −15° C., since this compound becomes too viscous to flow at −80° C. Tests with 2,2,4-trimethylpentane and the perfluorocyclic ether, $C_8F_{16}O$, showed that the recovered hydrocarbon contained less than 0.2% of the fluorochemical.

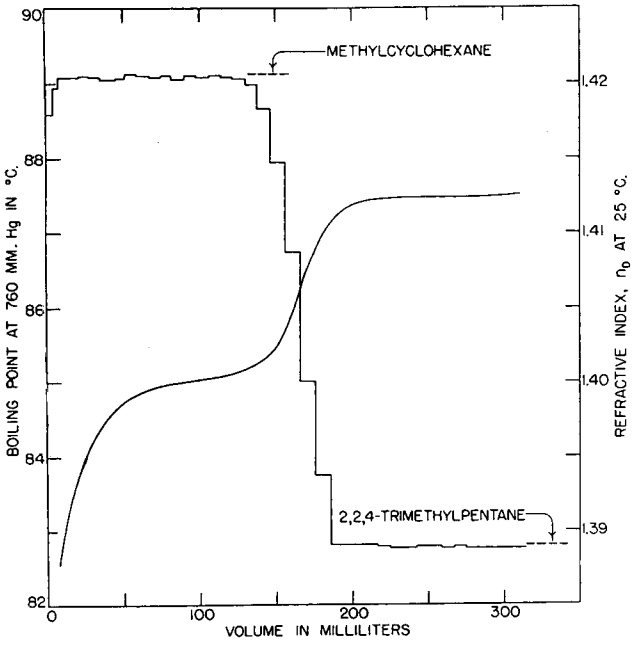

Figure 1. Azeotropic distillation of mixture of methyl cyclohexane and 2,2,4-trimethylpentane with perfluorocyclic ether, $C_8F_{16}O$

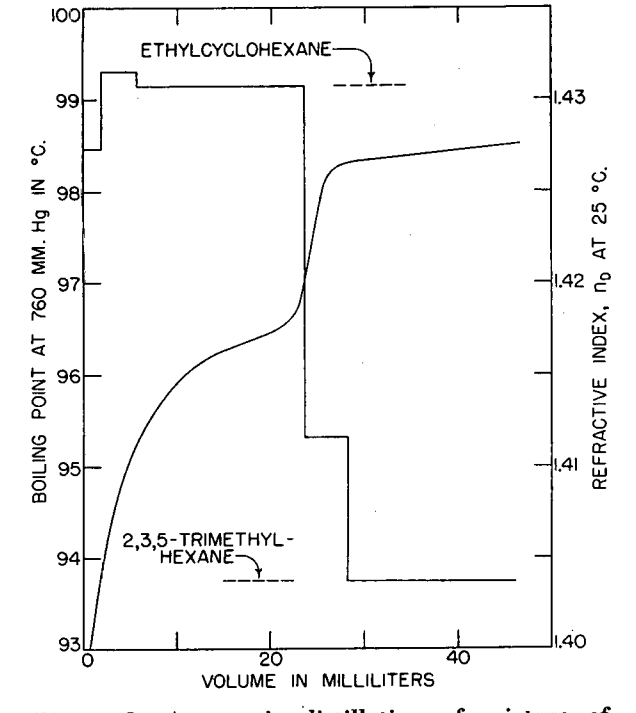

Figure 2. Azeotropic distillation of mixture of ethylcyclohexane and 2,3,5-trimethylhexane with perfluorocyclic ether, $C_8F_{16}O$

The results of the distillations of these three mixtures are shown, respectively, in Figures 1, 2, and 3. The distillations were performed in the standard manner of the API Research Project 6 at a reflux ratio of 200, in columns having a separating power about 200 theoretical plates at total reflux (*4*). The amount of fluorochemical used was that required to form the azeotropic mixture plus the amount retained as holdup in the column and pot (about 250 ml.).

Table I summarizes the data on these hydrocarbons and their azeotropic mixtures.

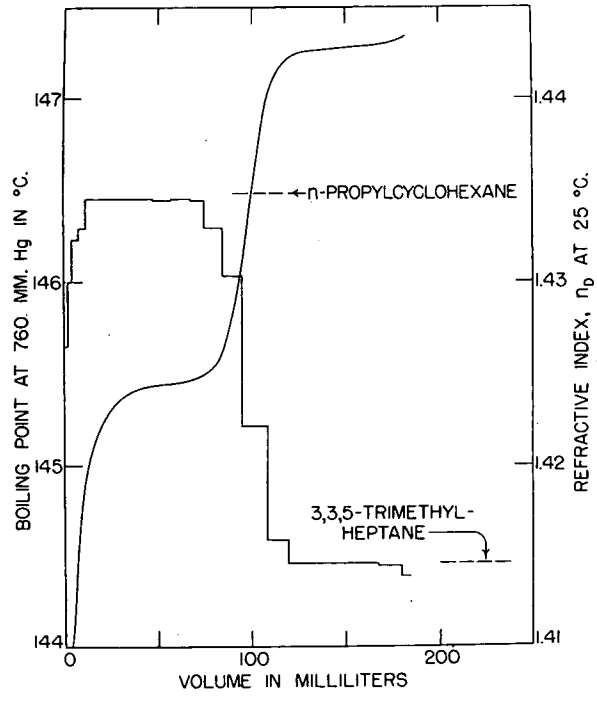

Figure 3. Azeotropic distillation of mixture of *n*-propylcyclohexane and 3,3,5-trimethylheptane with heptacosafluorotributylamine, $(C_4H_9)_3N$

In this work, data were obtained on the temperature of complete miscibility of binary equivolume mixtures of a number of hydrocarbons with each of four organic compounds—ethylene glycol monomethyl ether (methyl Cellosolve); diethylene glycol monomethyl ether (methyl Carbitol); perfluorocyclic ether $C_8F_{16}O$; and heptacosafluorotributylamine, $(C_4F_9)_3N$. The results are given in Figures 4 to 7.

With the two polar organic compounds (Figures 4 and 5), the individual hydrocarbons occupy nearly the same positions relative to one another in both cases, with the normal paraffin and branched paraffin hydrocarbons having higher temperatures of complete miscibility (lower solubilities) than the cycloparaffins.

With the two fluorochemical compounds (Figures 6 and 7), the individual hydrocarbons occupy the same positions relative to one another in both cases, with the cycloparaffins having higher temperatures of complete miscibility (lower solubilities) than the normal paraffins, which have higher temperatures of complete miscibility than the branched paraffins. From the

foregoing, the azeotropic distillation with fluorochemicals is concluded to be particularly effective for the separation of branched paraffins from cycloparaffins.

DISCUSSION

The data of this investigation show that the fluorochemicals, $C_8F_{16}O$, and $(C_4F_9)_3N$, form azeotropic mixtures with hydrocarbons of the proper volatility and that the boiling point of the azeotrope of a cycloparaffin with a given fluorochemical is lower than the corresponding azeotrope with a paraffin hydrocarbon, when the cycloparaffin and paraffin hydrocarbons have about the same normal boiling point. From the known data on polar organic compounds with respect to azeotropic mixtures with hydrocarbons, the boiling point of the corresponding azeotrope with an aromatic hydrocarbon of the same normal boiling point is assumed to be still lower.

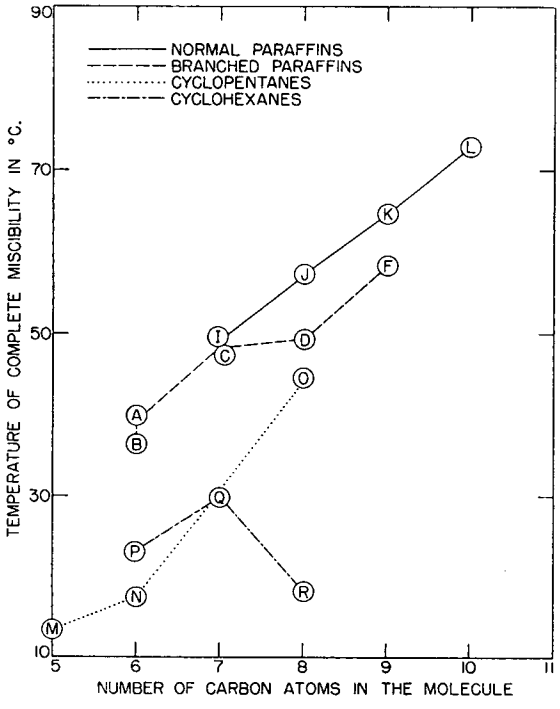

Figure 4. Temperatures of complete miscibility of binary equivolume mixtures of hydrocarbons with ethylene glycol monomethyl ether (methyl Cellosolve) plotted according to number of carbon atoms in hydrocarbon molecule

A. 2-Methylpentane
B. 3-Methylpentane
C. 2,4-Dimethylpentane
D. 2,2,4-Trimethylpentane
F. 2,2,5-Trimethylhexane
I. *n*-Heptane
J. *n*-Octane
K. *n*-Nonane
L. *n*-Decane
M. Cyclopentane
N. Methylcyclopentane
O. 1-Methyl-3-ethylcyclopentane (cis + trans)
P. Cyclohexane
Q. Methylcyclohexane
R. 1-*trans*-3-Dimethylcyclohexane

The lowering of the boiling point resulting from the formation of the cycloparaffin azeotrope (boiling point of the cycloparaffin hydrocarbon minus the boiling point of the cycloparaffin azeotrope) exceeds the lowering of the boiling point of the paraffin azeotrope (boiling point of the paraffin hydrocarbon minus the boiling point of the paraffin azeotrope) by a relatively large amount when a fluorochemical is used as the azeotrope-forming substance. For methylcyclohexane and 2,2,4-trimethylpentane, with the perfluorocyclic ether, $C_8F_{16}O$, as the azeotrope-forming substance, this excess lowering of the boiling point amounts to 4.2° C. The corresponding value for ethylcyclohexane and 2,3,5-trimethylhexane, with the same azeotrope-forming substance, is 2.5° C. For *n*-propylcyclohexane and 3,3,5-trimethylheptane, with heptacosafluorotributylamine, $(C_4F_9)_3N$, as the azeotrope-forming substance, the excess lowering of the boiling point is 2.9° C.

The unusual solubility characteristics of fluorochemicals have been recognized for some time and have been shown to fit within the theory of the solubility of nonelectrolytes developed by Hildebrand (*2*). As a part of this theoretical development, a quantity termed the "solubility parameter" was used to predict solubility in a semiquantitative manner. The solubility parameter, δ, is defined as the square root of the internal pressure or cohesive energy density of the pure substance

$$\delta = (\Delta E/V)^{1/2}$$

where ΔE is the energy of vaporization of the pure component and V is the molal volume of the liquid, all at the same temperature.

When the solubility parameters of the pure components of a binary mixture are the same, an ideal solution may be expected. When the solubility parameters of the components differ significantly, a nonideal solution, capable of forming azeotropes and of exhibiting limited solubility, may be expected. For the several classes of compounds discussed in this paper, the solubility parameters decrease in the following order: polar organic substances, aromatic hydrocarbons, cycloparaffin hydrocarbons, paraffin hydrocarbons, and perfluorinated compounds.

The lowering of the boiling point, which results when a binary azeotropic mixture is formed (boiling point of hydrocarbon minus boiling point of the azeotrope), depends on the magnitude of the deviation from ideality. Because the greater the difference in

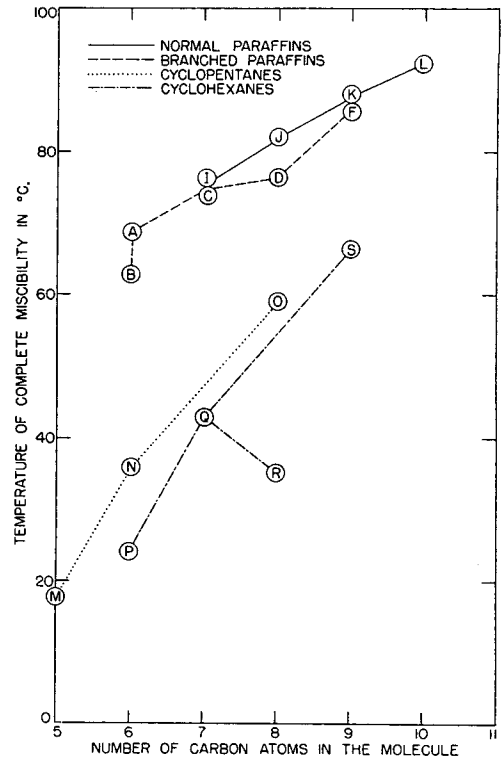

Figure 5. Temperatures of complete miscibility of binary equivolume mixtures of hydrocarbons with diethylene glycol monomethyl ether (methyl Carbitol)

Letters circled for hydrocarbons are listed under Figure 4

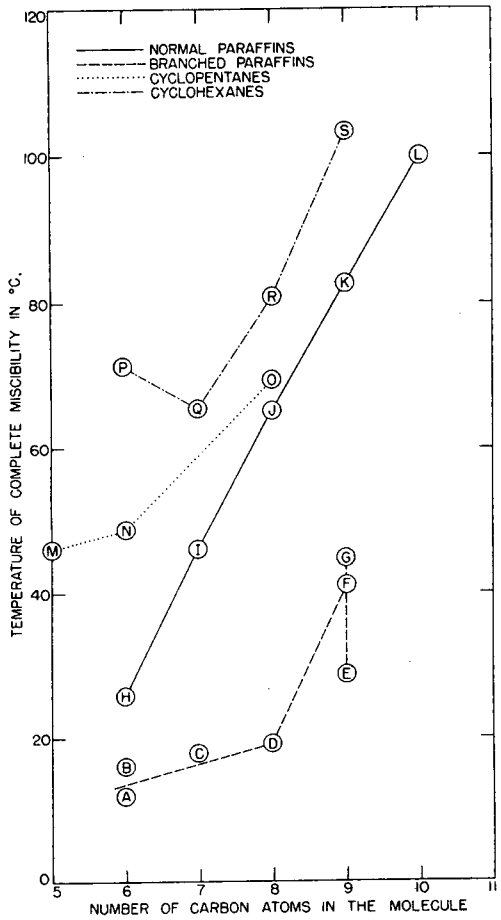

Figure 6. Temperatures of complete miscibility of binary equivolume mixtures of hydrocarbons with perfluorocyclic ether, $C_8F_{16}O$, plotted according to number of carbon atoms in hydrocarbon molecule

A. 2-Methylpentane
B. 3-Methylpentane
C. 2,4-Dimethylpentane
D. 2,2,4-Trimethylpentane
E. 2,2,4,4-Tetramethylpentane
F. 2,2,5-Trimethylhexane
G. 2,2,3,4-Tetramethylpentane
H. *n*-Hexane
I. *n*-Heptane
J. *n*-Octane
K. *n*-Nonane
L. *n*-Decane
M. Cyclopentane
N. Methylcyclopentane
O. 1-Methyl-3-ethylcyclopentane (cis + trans)
P. Cyclohexane
Q. Methylcyclohexane
R. 1-*trans*-3-Dimethylcyclohexane
S. *n*-Propylcyclohexane

solubility parameters of the components the greater the deviation from ideality, it is apparent that the lowering of the boiling points for binary azeotropic mixtures with a hydrocarbon as one component and a polar organic compound as the other component increases as the hydrocarbon component is changed in the following order: aromatic hydrocarbon, cycloparaffin hydrocarbon, and paraffin hydrocarbon. By the same reasoning, the lowering of the boiling point with a fluorochemical as one component increases as the hydrocarbon component is changed in the following order: paraffin hydrocarbon, cycloparaffin hydrocarbon, and aromatic hydrocarbon. The new experimental results obtained in this investigation with fluorochemicals as azeotrope-forming substances are in accord with the theoretical consideration.

For the separation of petroleum fractions containing branched paraffins and cycloparaffins by azeotropic distillation, the petroleum fractions should be prepared beforehand by regular distillation. Peaks in the plot of refractive index with respect to volume of distillate indicate the regions where cycloparaffins are being concentrated. These cycloparaffin concentrates contain branched paraffins boiling both above and below the cycloparaffin and also, in some cases, at almost exactly the same temperature. Azeotropic distillation with polar organic compounds is particularly effective for separating the lower boiling branched paraffins from the cycloparaffins (*4*). The reverse is true for azeotropic distillation with fluorochemical compounds. Thus, by azeotropic distillation, using alternatively the usual polar azeotrope-forming substances and the new fluorochemical azeotrope-forming substances, it is possible to effect a complete separation of branched paraffins from cycloparaffins. From the known data on the azeotropes of monocycloparaffins and dicycloparaffins (*4*) with polar organic compounds, the same procedure appears to be effective for the separation of monocycloparaffins from dicycloparaffins. The use of azeotropic distillation with fluorochemicals appears to offer the greatest promise of usefulness, at least on a laboratory scale, in connection with the two foregoing separations, where the methods heretofore used have not been entirely satisfactory.

In addition, the process may be used for the separation of aromatics from paraffins and cycloparaffins and for the separation of olefins from both aromatics and paraffins plus cycloparaffins. However, in these cases very good results may be obtained at less cost with ordinary polar organic compounds as azeotrope-forming substances. From Figures 6 and 7, the azeotropic distillation with fluorochemicals may be used to separate normal from branched paraffins of the same normal boiling point.

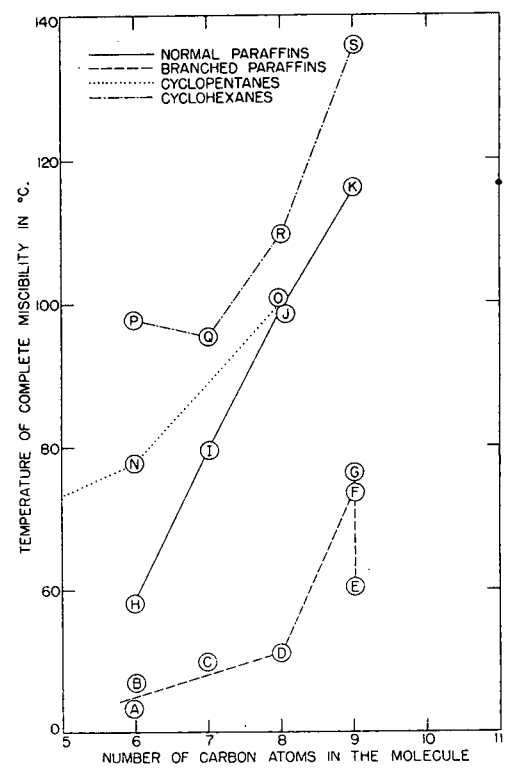

Figure 7. Temperature of complete miscibility of binary equivolume mixtures of hydrocarbons with heptacosafluorotributylamine, $(C_4F_9)_3N$, plotted according to number of carbon atoms in hydrocarbon molecule

Letters circled for hydrocarbons are listed under Figure 6

[This point has been established experimentally (*1*).] However, satisfactory results also can be obtained by other methods (*4*).

A similar reversal is apparent in the order in which hydrocarbons are separated using extractive distillation with fluorochemicals as compared with the usual polar substances and the discussion concerning the use of fluorochemicals in azeotropic distillation applies also to their use in extractive distillation.

The manner in which the change in structure of a fluorochemical affects its azeotrope-forming properties remains to be investigated. However, it is probable that the unique effect of fluorochemicals will be at a maximum with perfluorinated compounds and will diminish as fluorine atoms in the molecule are replaced by those of other elements.

ACKNOWLEDGMENT

The author desires to express his gratitude to Ned C. Krouskop for supervision of the distillation operations and to Michael D. Domenick for assistance in the experimental work. Acknowledgment is made to Frederick D. Rossini for his advice and encouragement.

LITERATURE CITED

(1) Cines, M. R., U. S. Patent **2,692,227** (Oct. 19, 1954).
(2) Hildebrand, J. H., and Scott, R. L., "Solubility of Nonelectrolytes," Reinhold, New York, 1950.
(3) Mair, B. J., Glasgow, A. R., Jr., and Rossini, F. D., *J. Research Natl. Bur. Standards* **27**, 39–63 (1941).
(4) Rossini, F. D., Mair, B. J., and Streiff, A. J., "Hydrocarbons from Petroleum," Reinhold, New York, 1953.

RECEIVED for review June 10, 1955. Accepted September 27, 1955. Division of Petroleum Chemistry, 128th Meeting, ACS, Minneapolis, Minn., September 1955. Investigation performed as part of the work of American Petroleum Institute Research Project 6 in the Petroleum Research Laboratory, Carnegie Institute of Technology, Pittsburgh, Pa.

Fractionation of Hydrocarbons by Adsorption with Added Components

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An improved method is described for separating hydrocarbons by adsorption, in which the fractionation takes place in the presence of two added components, one in the adsorbed phase and one in the liquid phase. Results are reported for experiments in which ethylene glycol monomethyl ether, or diethylene glycol monomethyl ether, constitutes the added component in the adsorbed phase, and heptacosafluorotributylamine, or perfluorocyclic ether constitutes the added component in the liquid phase. The method is particularly effective for the separation of branched paraffins from cycloparaffins.

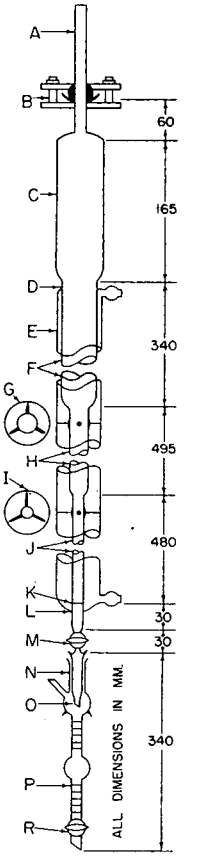
SINCE 1934, the American Petroleum Institute Research Project 6 has used fractionation by regular adsorption extensively in its work on the separation and purification of hydrocarbons. The process is particularly effective for the separation of aromatics from olefins, paraffins, and cycloparaffins, and for the separation of olefins from the other types of hydrocarbons (*3*, *5*).

This paper describes a new process for fractionating hydrocarbons by adsorption, in which the fractionation takes place in the presence of two added components, one in the adsorbed phase and one in the liquid phase. The process is an extension of the method of fractionation known as "partition chromatography," which was first reported by Martin and Synge (*4*) and is now used extensively in many fields.

METHOD

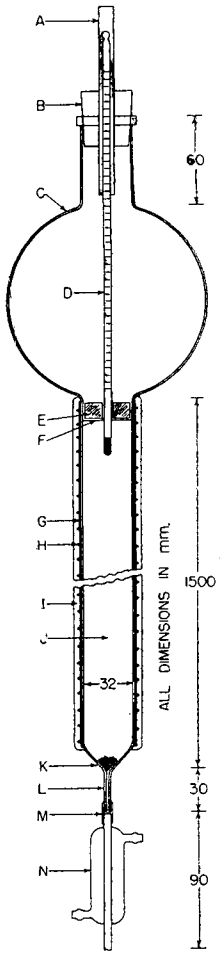
The adsorption column is packed with an adsorbent, which has been pretreated to contain the substance selected, to constitute the added component in the adsorbed phase. In the present experiments, either ethylene glycol monomethyl ether (methyl Cellosolve) or diethylene glycol monomethyl ether (methyl Carbitol) was used, although many other polar organic substances are suitable for this purpose. The liquid mixture of hydrocarbons to be separated is added to the adsorption column, and, after the mixture has completely entered the adsorbent, the material selected to constitute the added component in the liquid phase is introduced at the top of the column. In the present experiments either heptacosafluorotributylamine $(C_4F_9)_3N$ (Minnesota Mining and Manufacturing Co., No. N-43), or perfluorocyclic ether, $C_8F_{16}O$ (Minnesota Mining and Manufacturing Co., No. 0-75, believed to consist of a fluorinated side chain attached to a ring of five or six atoms, including oxygen) was used.

As the fluorochemical passes downward over the pretreated adsorbent, an interchange of hydrocarbon molecules takes place between the liquid phase composed largely of fluorochemical



A. Connection to source of inert gas pressure
B. Standard spherical joint (18/9)
C. Reservoir, 40 mm. in inside diameter
D. Heavy ring seal
E. Jacket sealed to rectifying section with inlet and outlet for passage of water
F. Upper part of fractionating section, 30 mm. in inside diameter
G. Three-pronged brace, slide fit and not sealed to jacket, prong 5 mm. in diameter
H. Middle part of fractionating section, 16 mm. in inside diameter
I. Three-pronged brace, slide fit and not sealed to jacket, prong 5 mm. in diameter
J. Lower part of fractionating section, 7 mm. in inside diameter
K. Sintered-glass plate of medium porosity
L. Heavy ring seal
M. Stopcock, 2-mm. bore with plug held firmly in place with spring clamp
N. Standard taper ground joint (14/35)
O. Tip of column
P. Receiver, graduated to 0.02 ml. in ranges 0 to 1.2 ml. and 4.5 to 5.5 ml.
R. Stopcock, 2-mm. bore

Figure 1. Glass adsorption column 1



A. Connection to source of inert gas pressure
B. Stopper
C. Reservoir, 1000-ml. capacity
D. Thermometer
E. Metal weight, coiled lead sheet
F. Glass cloth
G. Asbestos 1/16 inch in thickness
H. Electric resistance heating wire, asbestos covered
I. Corrugated sheet asbestos covered with aluminum foil, Alfol
J. Pretreated adsorbent
K. Glass wool
L. Capillary tube, 2-mm. bore
M. Tygon tubing
N. Condenser

Figure 2. Glass adsorption column 2

plus some hydrocarbon and the adsorbed phase composed largely of the glycol ether plus some hydrocarbon. The fluorochemical reduces the escaping tendency of the paraffin from the liquid phase relatively more than that of the cycloparaffin, whereas the glycol ether reduces the escaping tendency of the cycloparaffin from the adsorbed phase more than that of the paraffin. Thus, the two added components enhance each other so that the paraffinic component concentrates in the liquid phase, whereas the cycloparaffinic component concentrates in the adsorbed phase. As a result, the paraffin hydrocarbon issues from the column in advance of the cycloparaffin hydrocarbon. If the separation is reasonably complete, a plot of refractive index (or other suitable physical property) of the filtrate with respect to its volume shows two peaks. The first peak indicates the portion of filtrate containing the paraffinic component, whereas the second peak indicates the portion containing the cycloparaffin component.

Various procedures have been used for the recovery of hydrocarbons from their mixtures with the fluorochemical in the filtrate. Where the hydrocarbon and fluorochemical boil sufficiently far apart, they may be separated by distillation in a short column. However, if hydrocarbon and fluorochemical boil relatively close together (within about 60° C.), an azeotropic mixture is likely to be formed (*1*). Mixtures of fluorochemicals and hydrocarbons can usually be resolved simply by cooling to low temperatures and separating the two phases which are formed. At −80° C., with 2,2,4-trimethylpentane and the perfluorocyclic ether, the hydrocarbon phase contains abut 0.5% of the fluorochemical. Mixtures of 2,2,4-trimethylpentane, ethyl alcohol, and the fluorochemical, $C_8F_{16}O$, give two phases at −80° C., a phase largely fluorochemical and a phase largely alcohol plus hydrocarbon. By washing the alcohol-hydrocarbon phase with water, 2,2,4-trimethylpentane, containing less than 0.2% of fluorochemical, can be recovered.

APPARATUS AND PROCEDURE

It was found that the mixing of the adsorbent and added component was satisfactorily achieved in a Waring Blendor. The procedure for mixing was as follows: One milliliter of the selected solvent was placed in the blender to protect its bearings; a convenient quantity (70 grams) of adsorbent was then added; the blender was turned on, and the desired quantity of solvent added in 2- or 3-ml. portions. The amount of solvent added was the maximum volume the adsorbent could hold without becoming lumpy or sticky—that is, with the adsorbent remaining free-flowing. Mixing was continued until all lumps, which were temporarily produced, disappeared. Ordinarily, the time required for this operation was 5 minutes. The procedure causes a small reduction in particle size.

In addition to its use in preparing an adsorbent for the fractionating experiments, this procedure was also used to determine the volume of liquid a given weight of adsorbent could take up and still remain free-flowing. This was taken as the volume of liquid required to barely reach the point where the adsorbent changed from a free-flowing to a lumpy or wet state, and could usually be determined to within 2 ml. (liquid) per 100 grams of adsorbent. The volume of the adsorbed phase per unit mass of adsorbent may also be obtained by determining the gain in weight resulting from the equilibration of a known amount of adsorbent with the saturated vapors of various liquids (*2*) or from the difference between the apparent and true specific volumes of the adsorbent. The method used in this investigation is less accurate than the two foregoing methods, but is adequate for the purpose of rapidly screening adsorbents.

The two adsorption columns used in the work are shown in Figures 1 and 2. During the course of experiments with column 2, a portion of the pretreated adsorbent sometimes broke loose from the walls of the column, rose through the fluorochemical, and floated on its surface. To avoid this, a layer of glass cloth was placed on top of the adsorbent, and this, in turn, was held in position with a lead weight. Apart from this one modification, necessitated by the high density of the fluorochemicals, the procedure for packing the columns and conducting the experiments was similar to that given in previous reports (*5*).

Heptacosafluorotributylamine and perfluorocyclic ether were used as received. The ethylene glycol monomethyl ether was used as received. The diethylene glycol monomethyl ether was further purified by distillation.

Although a number of adsorbents were tested for capacity, only one adsorbent, silica gel, 100 to 200 mesh (Davison Chemical Corp., Grade 70) was used in the present experiments.

CAPACITY OF ADSORBENTS

The following results were obtained for the capacity of several adsorbents, in terms of the volume of liquid adsorbed (measured before adsorption) per unit mass of adsorbent: activated alumina (Aluminum Co. of America, Grade F-20) 0.23 ml. per gram; silicic acid (Mallinckrodt Chemical Co., chromatographic grade) 0.6 ml. per gram; silica gel (Davison Chemical Corp., Grade 922) through 200 mesh, 0.4 ml. per gram; silica gel (Davison Chemical Corp., Grade 70) 100 to 200 mesh, first lot, 0.9 ml. per gram, second lot, 0.7 ml. per gram.

EXPERIMENTS WITH ADDED COMPONENT IN ADSORBED PHASE ONLY

In this section results are given of experiments performed using an added component in the adsorbed phase only. In this case the hydrocarbon mixture alone is allowed to filter through the pretreated adsorbent containing the alcohol-ether compound. The

flow of the mixture of hydrocarbons is continued until material of the original composition issues as filtrate, so that all of the fractionation occurs in the material preceding this.

These experiments were performed at room temperature in column 1, packed with 155 grams of silica gel (Davison Chemical Corp., Grade 70), 100 to 200 mesh, plus 139 ml. of added component, either diethylene glycol monomethyl ether or ethylene glycol monomethyl ether. The results are given in Figures 3 to 6. The refractive indices plotted in these figures are those of the filtrate after washing with water in order to remove very small amounts of the added component.

Figure 3 shows the results of an experiment with an equivolume mixture of *n*-heptane and methylcyclohexane using diethylene glycol monomethyl ether as the added component. Three additional experiments, not shown, gave essentially identical results. Figure 4 shows the results of an experiment with an equivolume mixture of *n*-heptane and methylcyclohexane using ethylene glycol monomethyl ether as the added component.

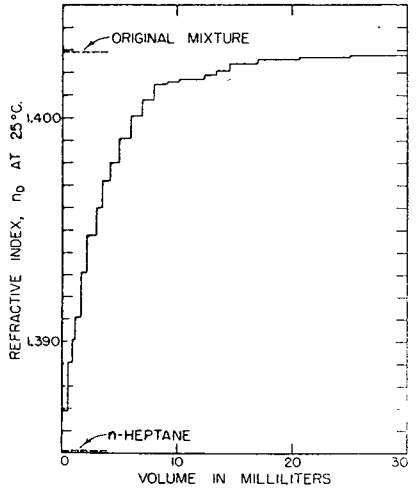


Figure 3. Fractionation of mixture of *n*-heptane and methylcyclohexane

Scale of ordinates gives refractive index, and scale of abscissas gives volume of hydrocarbon part of filtrate

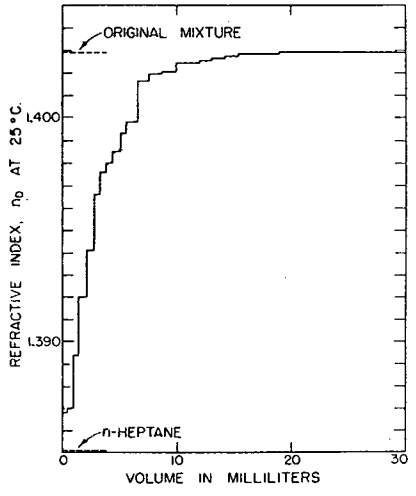


Figure 4. Fractionation of mixture of *n*-heptane and methylcyclohexane

See legend to Figure 3

Figure 5 shows the results of an experiment with an equivolume mixture of 2,4-dimethylpentane and cyclohexane using diethylene glycol monomethyl ether as the added component. Figure

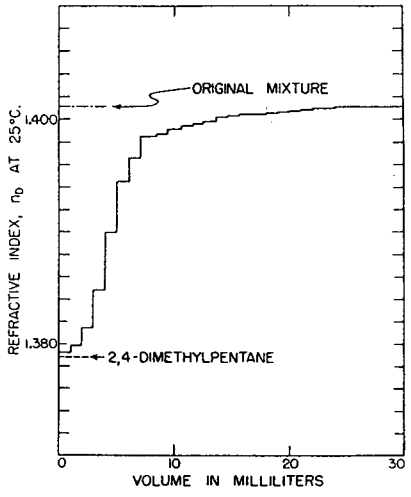


Figure 5. Fractionation of mixture of 2,4-dimethylpentane and cyclohexane

Legend same as for Figure 3

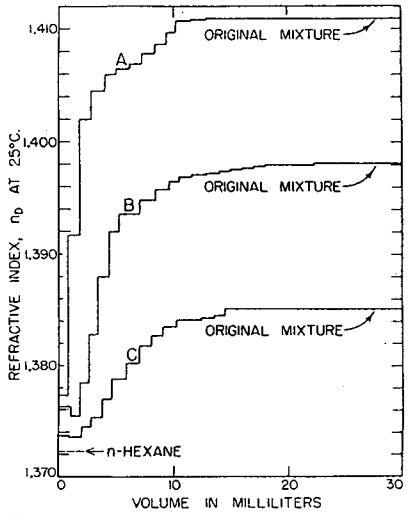


Figure 6. Fractionation of three mixtures of *n*-hexane and cyclohexane

See legend to Figure 3. Curves *A*, *B*, and *C* refer to solutions containing 25, 50, and 75 volume % *n*-hexane, respectively

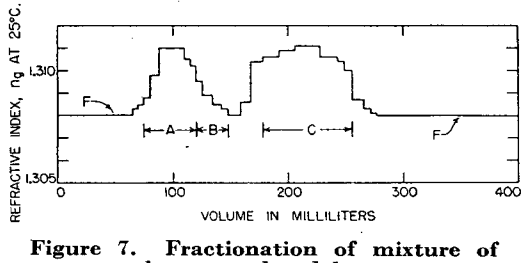


Figure 7. Fractionation of mixture of *n*-hexane and cyclohexane

See legend to Figure 8

6 shows the results of three experiments with mixtures of *n*-hexane and cyclohexane, using diethylene glycol monomethyl ether as the added component. The curves *A*, *B*, and *C* refer to solutions containing 25, 50, and 75 volume % *n*-hexane, respectively. From Figures 3 to 6 it is apparent that significant separations were obtained, with the first portion of the filtrate consisting of material greatly enriched in the paraffinic component.

In addition to the preceding experiments, one experiment was performed in column 1, using 167 grams of silicic acid (Mallinckrodt Chemical Co., chromatographic grade) pretreated with 100 ml. of diethylene glycol monomethyl ether. The separation was not as good as with the silica gel.

EXPERIMENTS WITH TWO ADDED COMPONENTS, ONE IN ADSORBED PHASE AND ONE IN LIQUID PHASE

In this section results are given of experiments in which small quantities (8 to 14 ml.) of binary mixtures of hydrocarbons were added to a column containing the adsorbent (silica gel, Grade 70) "loaded" with the added polar organic compound. These hydrocarbons, which were eluted with a fluorochemical, gave results as shown in Figures 7 to 14.

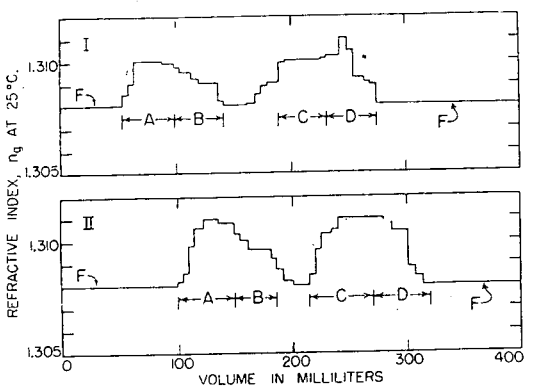


Figure 8. Fractionation of mixture of 2,4-dimethylpentane and cyclohexane

Scale of ordinates gives refractive index and scale of abscissas gives volume of filtrate. *F* denotes refractive index of fluorochemical. I gives results of first experiment, II gives results of a second experiment performed without repacking column

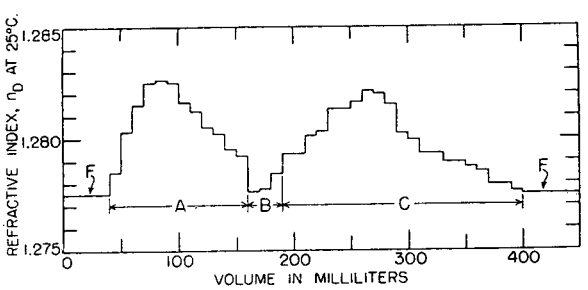


Figure 9. Fractionation of mixture of 2,2,4-trimethylpentane and methylcyclohexane

Legend same as for Figure 8

Figure 7 shows the results of an experiment with 8 ml. of an equivolume mixture of *n*-hexane plus cyclohexane. The experiment was performed in column 1 at 57° C., using 155 grams of silica gel pretreated with 139 ml. of diethylene glycol monomethyl ether. The hydrocarbon material was eluted with heptacosafluorotributylamine. Portions *A*, *B*, and *C* were distilled separately to recover the hydrocarbon. Substantially pure *n*-hexane was recovered from portions *A* and *B*, and substantially pure cyclohexane from portion *C*.

Figure 8 shows the results of two experiments with an equivolume mixture of 2,4-dimethylpentane plus cyclohexane. The first experiment, shown in the upper part of the figure, was performed with 8 ml. of the mixture in column 1 at 57° C., using 155 grams of silica gel pretreated with 139 ml. of diethylene glycol monomethyl ether. The hydrocarbon material was eluted with heptacosafluorotributylamine. The second experiment, shown in the lower part of the figure, was performed in exactly the same manner without repacking the column. In both experiments substantially pure 2,4-dimethylpentane was recovered by distillation from portions *A* and *B* and substantially pure cyclohexane from portions *C* and *D*. The results show that it is not necessary to repack the column after each experiment.

Figures 9 and 10 give the results of an experiment with an equivolume mixture of 2,2,4-trimethylpentane and methylcyclohexane. The experiment was performed with 14 ml. of the mixture in column 2 at 50° C., using 480 grams of silica gel pretreated with 336 ml. of diethylene glycol monomethyl ether. The hydrocarbon material was eluted with the perfluorocyclic ether. Portions *A*, *B*, and *C* were extracted separately with ethyl alcohol at −80° C. to recover the hydrocarbon material. Figure 10 shows the excellent results obtained.

Figures 11 and 12 give the results of an experiment with 14 ml. of an equivolume mixture of *n*-heptane and methylcyclohexane. The experiment was performed at 50° C. after the ex-

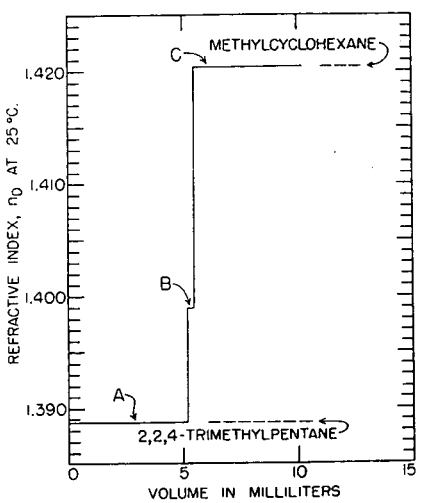


Figure 10. Fractionation of mixture of 2,2,4-trimethylpentane and methylcyclohexane

See legend to Figure 3. Results are from same experiment as for Figure 9

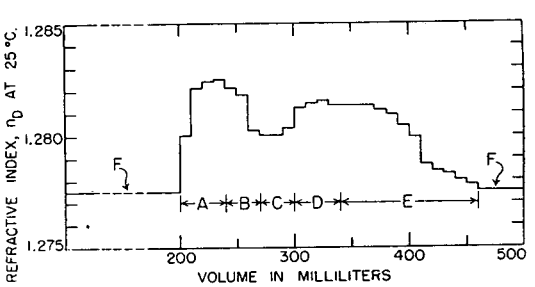


Figure 11. Fractionation of mixture of *n*-heptane and methylcyclohexane

Legend same as for Figure 8

periment shown in Figure 9, without repacking the column. The hydrocarbon material was eluted with the perfluorocyclic ether. Portions *A*, *B*, *C*, *D*, and *E* were extracted separately with ethyl alcohol at −80° C. to recover the hydrocarbon material. Figure 12 shows the excellent results obtained.

Figures 13 and 14 give the results of an experiment with an equivolume mixture of 2,2,5-trimethylhexane and *n*-propylcyclohexane. The experiment was performed with 14 ml. of the mixture in column 2 at 67° C. using 492 grams of silica gel pretreated with 336 ml. of diethylene glycol monomethyl ether. The hydrocarbon material was eluted with the perfluorocyclic ether. Portions *A*, *B*, *C*, *D*, *E*, *F*, and *G* were extracted separately with ethyl alcohol at −80° C. to recover the hydrocarbon material. Figure 14 shows the excellent separation obtained.

DISCUSSION OF RESULTS

The amount of separation which can be obtained depends (other things being equal) on the amount of interchange between the phases, and this depends on the volume of the phases. Thus, to keep the size of the apparatus at a minimum, it is desirable to select an adsorbent for which the volume of the adsorbed phase per unit volume of adsorbent is as large as possible. It was for this reason, that silica gel, 100 to 200 mesh (Davison Chemical Corp., Grade 70), was used for the experiments shown in Figures 3 to 14, inclusive.

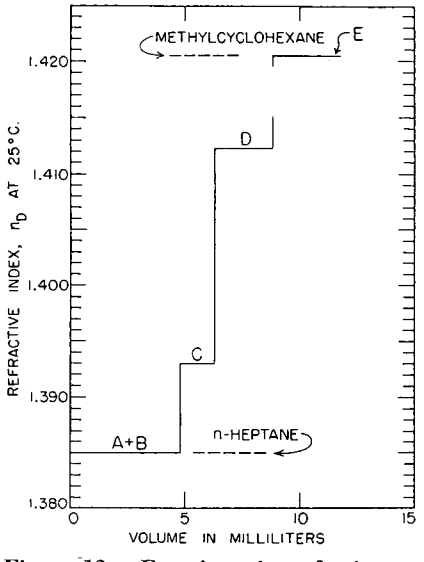


Figure 12. Fractionation of mixture of *n*-heptane and methylcyclohexane

See legend to Figure 3. Results are from same experiment as for Figure 11

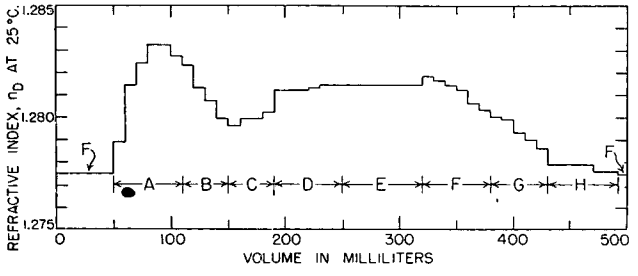


Figure 13. Fractionation of mixture of 2,2,5-trimethylhexane and *n*-propylcyclohexane

See legend to Figure 8

The experiments given in Figures 3 to 6 with the added component in the adsorbed phase only, indicate that significant separations of paraffin from cycloparaffin hydrocarbons can be obtained using either diethylene glycol monomethyl ether or ethylene glycol monomethyl ether. In all cases, the paraffin hydrocarbon is concentrated in the first portion of the filtrate. This is the reverse of what frequently happens in regular adsorption; for example, with an equivolume mixture of cyclohexane and *n*-hexane the initial portion of the filtrate is enriched in cyclohexane (*5*).

The experiments displayed in Figures 7 to 14 show that almost complete separations of paraffins from cycloparaffins are obtained by adsorption with two added components, one in the adsorbed phase and one in the liquid phase. With a greater ratio of mass of adsorbent to volume of charge, it is expected that quantitative separations can be achieved.

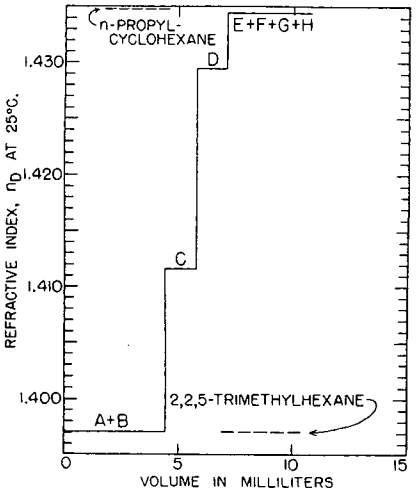


Figure 14. Fractionation of mixture of 2,2,5-trimethylhexane and *n*-propylcyclohexane

Legend same as for Figure 3. Results are from same experiment as for Figure 13

A comparison of the results for the separation of 2,2,4-trimethylpentane from methylcyclohexane, as shown in Figures 9 and 10, with those for *n*-heptane from methylcyclohexane, as shown in Figures 11 and 12, shows a more complete separation of the 2,2,4-trimethylpentane. This is to be expected, since 2,2,4-trimethylpentane is more soluble than *n*-heptane in the fluorochemical (*1*). Branched paraffins, in general, are more soluble in fluorochemicals than the corresponding normals, and, as a consequence, the separation of the branched paraffins from cycloparaffins may be effected more readily than that of normal paraffins from cycloparaffins.

It was discovered early that operation at room temperature did not give satisfactory results in many cases. The solubilities of the hydrocarbon components were too low at this temperature, and the volume of fluorochemical required to elute them from the column was too large to be practicable. As a working rule, satisfactory results were obtained by operating at or slightly above the temperature of complete miscibility of the paraffin in the fluorochemical. It would seem that operation at still higher temperatures is practicable, if the volatility of the various components permits, and if the solubility of the fluorochemical and glycol ether in each other do not become too great.

It is important that the hydrocarbon components be distributed approximately equally between the adsorbed and the liquid

phases. If the hydrocarbon is nearly all in the adsorbed phase, very large volumes of fluorochemical are required to elute it. If, on the other hand, the hydrocarbon is nearly all in the liquid phase little interchange takes place and the hydrocarbon passes from the column without being significantly separated. Satisfactory results are obtained if the solubility of the paraffinic hydrocarbon in the fluorochemical is of the same order of magnitude as that of the cycloparaffin hydrocarbon in the added component in the adsorbed phase. Temperatures of complete miscibility, which are useful in comparing solubilities of hydrocarbons in the fluorochemicals and glycol ethers discussed here, are given by Mair (*1*).

While only paraffins and cycloparaffins have been included in the experiments reported here, there is little doubt that many other types of hydrocarbons may be separated by this method. Other separations include those of aromatics and olefins from each other and their separation from paraffins and cycloparaffins. Since, however, aromatics and olefins can be separated readily by other methods including regular adsorption, the most important use of the new method appears to be for the separation of paraffins, particularly branched paraffins, from cycloparaffins. From the known solubilities of monocycloparaffins and dicycloparaffins in the usual polar solvents, and their estimated solubilities in fluorochemicals, it is predicted that another important application will be for the separation of monocycloparaffins from dicycloparaffins.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Mair, B. J., ANAL. CHEM. **28**, 52 (1956).
(2) Mair, B. J., Westhaver, J. W., and Rossini, F. D., *Ind. Eng. Chem.* **42**, 1279 (1950).
(3) Mair, B. J., and White, J. D., *J. Research Natl. Bur. Standards* **15**, 51 (1935).
(4) Martin, A. J. P., and Synge, R. L. M., *Biochem. J.* (*London*) **35**, 1358 (1941).
(5) Rossini, F. D., Mair, B. J., and Streiff, A. J., "Hydrocarbons from Petroleum," Reinhold, New York, 1953.

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Determination of Cobalt by Anodic Electrodeposition

Utilization of Isotope Dilution Method

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Cobalt may be satisfactorily determined by an anodic electrodeposition–isotope dilution method. Since the isotope dilution technique requires that only a weighable portion of the pure oxide be deposited on the anode, the time of electrolysis may be short, and adherence difficulties that are encountered with cobalt oxide deposits are eliminated as a source of error. The weighing form of the deposit is cobaltic oxide trihydrate.

ALMOST since the beginning of electroanalytical work it has been known that cobalt and nickel may be deposited anodically as oxides (*2*, *14*). Several investigators have indicated the possibility of utilizing anodic behavior in the separation (*1*, *11*) and the determination (*9*, *11*) of cobalt. From the standpoint of their routine use in analysis, previous analytical methods involving the anodic deposition of cobalt as an oxide have not been satisfactory. Quantitative separations of cobalt from nickel were obtained only by going to such extremes as using 10 platinum anodes interchangeably over a 20-hour period (*1*), or by resorting to double deposition of the oxide at elevated temperatures and using separated electrode compartments (*11*). In addition there is a definite tendency for the deposits to become nonadherent as electrolysis proceeds.

In the method given in the present paper, which involves both anodic electrodeposition and the isotope dilution technique (*10*), the duration of electrolysis can be decreased considerably. In addition, nonadherence of the deposit is eliminated as a source of error, because the very thin deposits that are used adhere well to the electrode, and, even though some of the deposit is lost from the anode before the weighing and counting operations, no error is introduced.

Cobalt-60, a beta-gamma emitter having a half life of 5.3 years, served as the radioactive tracer for the isotope dilution. Highly pure radioactive cobalt can be obtained from Oak Ridge at a low cost.

METHOD

A series of solutions containing various known weights of pure cobalt as sulfate was prepared. A fixed volume of an active cobalt solution was added to each, the solution was buffered, and deposits were plated on sandblasted platinum disk anodes, using a modified tower electrolysis cell (*10*). Each deposit was dried, weighed and counted, and its specific activity, *S.A.*, calculated in counts per minute per milligram. If W, the weight of inactive cobalt taken, is plotted against $1/S.A.$, a linear standard curve is obtained which obeys the equation

$$W = A\left(\frac{1}{S.A.}\right) - w$$

where A is the total activity of the volume of active cobalt used for the isotope dilution, and w is the weight of cobalt in the added active cobalt solution.

When cobalt occurs in solution with other metal ions, many of them precipitate as hydroxides, hydrous oxides, or sulfates as the solution is being buffered to pH 7.8 for the anodic deposition. If the amount of the other metal is not too large, this precipitate can simply be filtered off and cobalt deposited as usual from the filtrate. The quantity of metal or metals to be separated in this manner is limited only by the fact that sufficient cobalt must remain unadsorbed in the solution to form a weighable deposit on the anode during subsequent electrodeposition.

If, on the other hand, substances are present which are not precipitated below a pH of 7.5 to 8.0, they may or may not interfere with deposition. Those expected to interfere include: cations

known to deposit anodically—e.g., manganese, nickel, lead, and silver—substances strongly adsorbed on hydrous oxides—e.g., arsenious acid (*13*)—and reducing agents such as organic anions (*4*) and acids (*8, 9*), halides, hydrogen peroxide, etc.

APPARATUS

Modified Tower Electrolysis Cells, 50 ml. (*10*)
Platinum Disk Anodes, $^{15}/_{16}$ inch in diameter × 0.005 inch thick, with one surface uniformly sandblasted
Sample Changer, Tracerlab SC-9D shielded, manual
Scaler, Potter predetermined decade scaler, Model 341
Geiger Tube, Tracerlab TGC-2
Electroanalyzer, Eberbach, rotating electrode
Microbalance
Drying Oven, adjustable to 40° C.
Aluminum Absorber, 70 mg. per sq. cm.
pH Meter, Beckman Model H2
Clinical Centrifuge, 50-ml. borosilicate glass centrifuge tubes

REAGENTS

Unless otherwise stated all chemicals were of the analyzed reagent type. Double distilled water was used in preparing all solutions.

Radioactive Cobalt Solution. Cobalt-60 was purchased from the U. S. Atomic Energy Commission, Isotopes Division, Oak Ridge, Tenn. (ORNL). Approximately 1 mc. of this solution with 200 mg. of carrier was diluted to 2 liters.

Standard Cobalt Solution. In the minimum amount of concentrated sulfuric acid 1.000 gram of spectrographically pure cobalt sponge was dissolved, and then diluted to 1 liter. Matthey cobalt sponge of Johnson, Matthey and Co., Ltd., London, was used.

Boric Acid–Potassium Sulfate Buffering Solution. One liter of a solution that was 0.10*M* in boric acid and 0.05*M* in potassium sulfate was prepared. To adjust the pH to the desired value 0.1 and 0.5*M* sodium hydroxide solutions were used.

Solutions of metal ions for interference and separation studies were prepared from the nitrates, sulfates, or oxides. Each solution contained 2 mg. per ml. of metal. Oxides were initially dissolved in the minimum amount of sulfuric acid and then diluted.

PROCEDURE

Preparation of Standard Curve. Pipet 10-ml. portions of the radioactive cobalt solution into various volumes of the standard inactive cobalt solution. Mix thoroughly. Add 20 ml. of boric acid–potassium sulfate solution, and add 0.1*M* sodium hydroxide dropwise with stirring until the pH is 7.6 to 7.8, as determined with a pH meter. Dilute to 50 ml. with double distilled water. If a small precipitate of basic salt forms, filter the solution and discard the precipitate. Clean the sandblasted platinum disk anode (using acid iodide or oxalate to remove oxide deposits), dry for 15 minutes at 40° C., and weigh to the nearest 0.002 mg. With the disk in place in the cell, introduce the active buffered solution. Electroplate at 1.5 to 1.8 volts for 40 minutes. Remove the still active solution through the cell's glass side arm, rinse out the cell with double distilled water, and remove the disk which now contains the oxide deposit. Wash the deposit with double distilled water and remove adhering water droplets with a piece of filter paper. Allow the disk to dry in air until no water is visible, and place in a 40° C. oven for 2 to 2.5 hours. Weigh as before. Determine the activity of the deposit by placing the disk in a planchet in position in the sample changer with a 70-mg. per sq. cm. aluminum absorber in place. Make dead time, background, and efficiency corrections on the observed activity, and calculate the specific activity.

Data for a standard curve are given in Table I. At *W* values less than 6 mg., the small amount of deposit formed in 40-minute deposition leads to some uncertainty. Similarly, above about 15 mg., the activity of deposits obtained in the usual electrodeposition time is low; this also leads to uncertainty or inconvenience. Consequently, the portion of the curve between 5 and 15 mg. is best for most purposes and should be used if the approximate per cent cobalt in an unknown is known.

Counting only the gamma radiation from deposits removes the possibility of errors due to self-absorption of the beta radiation. The 70-mg. per sq. cm. aluminum absorber is suitable to screen out the beta rays. A standard gamma source that was counted daily and used in maintaining constant counting efficiency consisted of a sample of active metallic cobalt deposited on a disk electrode like those used for the oxide deposits.

Table I. Preparation of Standard Curve

Wt. of Co, Mg.	*S.A.* of Oxide Deposit, Counts Per Min./Mg.	Av.	$1/S.A.$, Mg./Counts/Min.
5.000	2755 2655	2705	3.697×10^{-4}
7.500	2042 1975 1986	2001	4.998×10^{-4}
10.00	1558 1542 1552	1551	6.448×10^{-4}
12.50	1226 1192 1242	1220	8.196×10^{-4}
15.00	1055 1060 1049	1055	9.479×10^{-4}
20.00	790 812	801	12.5×10^{-4}

Table II. Interference Study

Initial Composition of Soln.	$1/S.A.$	*W*, Mg.	% Error
A. Noninterfering substances			
10.00 mg. of Co	6.375×10^{-4}	9.720	−2.8
10.00 mg. of Co	6.520×10^{-4}	9.980	−0.2
10.00 mg. of Co + 10.0 mg. of Fe	6.470×10^{-4}	9.970	−0.3
10.00 mg. of Co + 10.0 mg. of Cu	6.520×10^{-4}	9.980	−0.2
10.00 mg. of Co + 10.0 mg. of Fe + 10.0 mg. Cu	6.635×10^{-4}	10.15	+1.5
10.00 mg. of Co + 10.0 mg. of Cr as $Cr_2O_7^{--}$	6.560×10^{-4}	10.03	+0.3
10.00 mg. of Co + 10.0 mg. of Zn	6.580×10^{-4}	10.07	+0.7
10.00 mg. of Co + 10.0 mg. of Bi	6.450×10^{-4}	9.860	−1.4
10.00 mg. of Co + 10.0 mg. of Ag	6.580×10^{-4}	10.07	+0.7
10.00 mg. of Co + 10.0 mg. of Al	6.455×10^{-4}	9.880	−1.2
10.00 mg. of Co + 10.0 mg. each of Ba, Ca, Sr, Mg	6.720×10^{-4}	10.30	+3.0
10.00 mg. of Co + 10.0 mg. of Pb	6.550×10^{-4}	10.02	+0.2
10.00 mg. of Co + 10.0 mg. of Cd	6.565×10^{-4}	10.03	+0.3
B. Interfering substances			
10.00 mg. of Co + 10.0 mg. of Ni	6.940×10^{-4}	10.69	+6.9
10.00 mg. of Co + 10.0 mg. of Ni[a]	7.190×10^{-4}	11.06	+10.6
10.00 mg. of Co + 10.0 mg. of Mn	64.5×10^{-4}	ca. 109	>1000
10.00 mg. of Co + 10.0 mg. of As	9.970×10^{-4}	15.91	59
10.00 mg. of Co + 10.0 mg. of Hg (acetate)	7.455×10^{-4}	11.56	15.6
10.00 mg. of Co + 10.0 mg. of Hg (oxide)	9.40×10^{-4}	14.8	48
C. Separation of Co and Mn by cobaltinitrite pptn.			
10.00 mg. Co + 10.0 mg. of Mn (1 pptn.)	6.589×10^{-4}	10.10	+1.0
10.00 mg. Co + 10.0 mg. of Mn (2 pptns.)	6.490×10^{-4}	9.94	−0.6
D. Separation of Co and Hg by use of Cu			
10.00 mg. Co + 10.0 mg. of Hg (oxide)	6.700×10^{-4}	10.28	+2.8

[a] At pH of 5 and at 90° C., according to Torrance (*11*).

Separations and Interference Study. To a mixture of 10.00 ml. of standard cobalt solution, 10 ml. of radioactive cobalt, and 20 ml. of buffer, add 10.0 mg. of the metal to be tested. Add 0.5*M* sodium hydroxide to a pH sufficient to precipitate the added metal as hydroxide (or to 7.6 to 7.8 if no precipitate forms). Filter off the precipitate on a coarse filter paper. Adjust to pH 7.6 to 7.8, filtering again if necessary. Proceed with the electrodeposition in the described manner. Find the specific activity as before, and read the weight of cobalt from the standard curve. During the buffering process, iron is precipitated as ferric hydroxide at a pH of 3.5. Cupric, zinc, aluminum, and cadmium ions are removed by filtration after precipitation at pH 6.5. Lead, alkaline earths, and some

silver ions precipitate as sulfates and are filtered off.

Table II, A, shows that several metals, when initially present in an amount equal to that of the cobalt, do not interfere with the determination. Table II, B, shows results for ions from which deposition separation may not be made and which must be separated from cobalt prior to deposition by some method other than hydroxide or sulfate precipitation. It was already known that nickel codeposits to a small extent (*11*).

Cations codepositing were manganese and nickel, manganese being by far the worse. The cobaltinitrite method of precipitation of cobalt can be used to effect a separation of these metals prior to anodic deposition. Arsenite or arsenious acid strongly inhibits deposition probably because of the fact that it is adsorbed on the surface of the deposit that does form. Because ferric iron, precipitated at pH 3 from a hot solution, strongly adsorbs the arsenic, the arsenic can be removed from cobalt-arsenic mixtures leaving most of the cobalt in an arsenic-free filtrate. Mercury can be removed by adding finely divided copper to the acidic mixture, shaking intermittently for 15 minutes, and decanting (Table II, D).

For the determinations of Table II, A, C, and D, the standard deviation of a single measurement is 1.48%

Table III. Comparison of Plating Methods[a]

Expt. No.	Soln. Used	Voltage	Description of Deposit	Behavior during Plating	Cathode Deposit	Reference
1	0.05 g. $K_2Cr_2O_7$	2.3	Very thin, red-brown	No change	None	(*1*)
2	40 ml. 30% NaOH	0.5	Nonadherent, dark brown to black	Soln. changed from deep blue to gray; black suspension	Slight	(*3*, 12)
3	0.5 g. $K_2C_4H_4O_6$ 5 ml. 30% NaOH	2.0	Good and uniform, jet black	Soln. turned yellow-green to tan	None	(*6*, *14*)
4	30 ml. 30% NaOH 1 ml. glycerol	1.5	Nonuniform, brown	No change decomposed on standing	Yes	(*12*)
5	0.5 ml. $HC_2H_3O_2$ $NaC_2H_3O_2$ to pH 5	Up to 5	No deposit	No change	Yes, dark	(*11*)
6	0.05 g. $K_2Cr_2O_7$ 0.3 g. K_2SO_4	2.3	Black forms slowly	No change	None	(*1*)
7	20 ml. 0.1*N* NaOH 20 ml. 0.1*M* NaH_2PO_4	Up to 2	Nonuniform	Pink precipitate forms in soln.	None	
8	3 ml. 0.1*N* NaOH 25 ml. 0.1*M* H_3BO_3 in 0.05*M* K_2SO_4; pH = 7.5	1–2	Uniform, brown-black plate fairly adherent	No change	None	
9	$NaHCO_3$ to neutral, 0.5 g. excess	Up to 2	Good black deposit, very adherent	Soln. turned light green, no ppt.	None	

[a] Following factors affecting type and quality of deposits were held constant throughout study; temperature, 25° C.; deposition time, 20 minutes; amount of cobalt used, 10 mg. as sulfate; volume of plating bath was 50 ml.; smooth platinum anodes were used.

Table IV. Reproducible Deposits

(Deposited from borate buffer at pH 7.6, 1.7 volts, and 1 to 2 ma. per sq. cm dried 2.5 hours at 40° C.)

Wt. of Deposit, Mg.	Activity Net C.P.M.	*S.A.* C.P.M./ Mg.	% Co = $\frac{S.A.\ \text{Deposit}}{S.A.\ \text{Metal}} \times 100$
3.294	5438	1651	53.9
3.283	5426	1652	53.9
2.690	4373	1626	53.1
2.301	3867	1680	54.8
2.020	3316	1642	53.6
2.645	4331	1638	53.5
1.841	2991	1625	53.0

S.A. of metal = 3064 c.p.m./mg. Av. % Co = 53.68

REPRODUCIBLE DEPOSITION

Before undertaking the development of the analytical method, a study was made to determine the conditions under which a reproducible weighing form of the oxide could be obtained. The results are shown in Table III. Plates obtained in experiments three, eight, and nine appeared to be best. Deposition from solutions buffered with borate was chosen for subsequent experiments.

Two means of quantitatively studying reproducibility were used: the specific activity of anode deposits obtained under the same set of conditions, and the per cent cobalt in the deposits. The per cent cobalt was calculated by the following equation:

$$\% \text{ Co} = \frac{\text{specific activity of oxide}}{\text{specific activity of metal}} \times 100$$

The same active cobalt was used in the preparation of both metal and oxide deposits on the same type of disk electrodes. Identical geometry conditions were used in counting.

The following procedures for drying the oxides were tested: at room temperature in a desiccator over anhydrous calcium sulfate; at room temperature in a vacuum desiccator over barium oxide; at several temperatures from 30° to 175° C. in an oven; and finally, ignition at 300°, 500°, and 1000° C. Drying for 2.5 hours at 40° C. gave the best reproducibility. Ignition to cobalto-cobaltic oxide, as suggested by Smith (*9*), led to deposits which were very difficult to strip.

The results of activity measurements for a series of deposits prepared from solutions buffered with boric acid–borate and dried at 40° C. are shown in Table IV. The absolute standard deviation for these experimental values of the per cent cobalt is 0.41%. Thus, the reproducibility is satisfactory and well within the limitations of the isotope dilution method.

COMPOSITION OF DRIED DEPOSITS

The experimental value of 53.68% cobalt (Table IV) that was obtained from activity measurements indicates that the composition of the dried deposits is cobaltic oxide trihydrate (theoretical % cobalt = 53.60). This is in agreement with other findings (*1*, *5*, *7*).

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LITERATURE CITED

(1) Coehn, A., and Glaser, M., *Z. anorg. Chem.* **33**, 9 (1903).
(2) Fischer, N. W., *Kastn. Arch.* **16**, 219 (1829); *Pharm. Centr.* 109-10 (1830).
(3) Grube, G., and Feucht, O., *Z. Elektrochem.* **25**, 568 (1922).
(4) Haissinsky, M., *J. chim. phys.* **29**, 469 (1932).
(5) Huttig, G. F., and Kassler, R., *Z. anorg. Chem.* **184**, 284 (1929).
(6) Muller, E., and Spitzer, F., *Ibid.*, **50**, 326–7 (1906).
(7) Root, J. E., *J. Phys. Chem.* **9**, 1 (1905).
(8) Scholl, G. P., *J. Am. Chem. Soc.* **25**, 1049 (1903).
(9) Smith, E. F., *Trans. Am. Electrochem. Soc.* **27**, 30–4 (1915); "Electroanalysis," 6th ed., pp. 137, 146, Blakiston, Philadelphia, 1918.
(10) Theurer, K., and Sweet, T. R., ANAL. CHEM. **25**, 119 (1953).
(11) Torrance, S., *Analyst* **64**, 109 (1939).
(12) Tubandt, C., *Z. anorg. Chem.* **45**, 368 (1905).
(13) Weiser, H. B., "Inorganic Colloid Chemistry," vol. 2, p. 36, Wiley, New York, 1935.
(14) Wernicke, W., *Pogg. Ann.* **141**, 109 (1870).

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Characterization of Starch and Related Polysaccharides by Differential Thermal Analysis

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Differential thermal analysis was investigated as a technique for characterizing starches which showed thermal properties dependent upon the method of preparation. Distinct variations were observed among the amylose and amylopectin fractions. A tentative interpretation of the thermograms is given. The analysis has been extended to glycogen, cellulose, and dextran. The results suggest that differential thermal analysis is a promising technique for the characterization of polysaccharides and should find further useful applications in the difficult field of natural and synthetic high polymers.

DIFFERENTIAL thermal analysis, frequently used for the examination of inorganic systems, has been adapted for the characterization of certain organic substances (*4, 12, 22*). The method consists of heating, at a constant rate, an admixture of the organic material in calcined alumina. The temperatures at which the organic material undergoes exothermic and endothermic reactions are electronically recorded and measured with reference to a thermally inert material—for example, alumina.

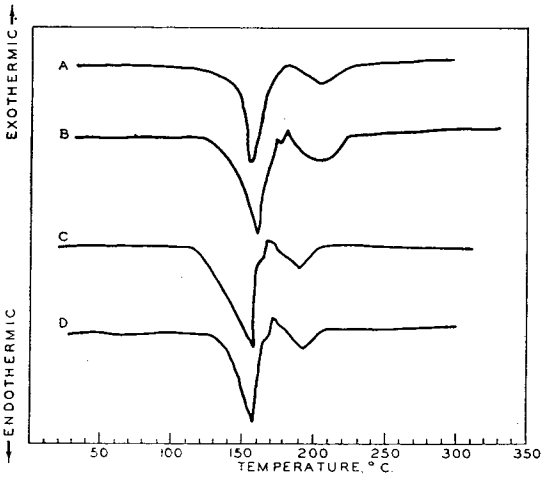

Figure 1. Thermograms of glucose isomers

A. α-Glucose
B. 50 to 50 mixture of α- and β-glucose
C. β-Glucose
D. β-Glucose duplicate run

The temperatures and the magnitude of these pyrolysis reactions have been found to be characteristic of each substance studied. Consequently the thermal curves may be used as a "fingerprinting" device for the identification of organic compounds. Moreover, the technique is suited to the analysis of insoluble colloidal or amorphous solids which cannot be examined conveniently by other instrumental procedures.

Tentative results (*12*) showed that the differential thermal features exhibited by many natural high polymers depended not only upon their chemical composition, but also upon their macromolecular configuration. Studies of a series of starch fractions and certain related polysaccharides are presented to illustrate further the versatility and the potentiality of the technique.

Important contributions to our knowledge concerning the structure and properties of starch and similar carbohydrates have been made by a variety of physicochemical studies (*1, 8, 11, 15, 16, 23*). Inasmuch as they are, for the most part, based on certain properties of their solutions, the successful adaptation of this technique would circumvent the difficulties encountered in attempts to prepare and use stable soluble derivatives (*10, 11*). However, it must be recognized that the sensitivity and precision attainable are such that only tentative supplementary data, which must be confirmed by other established criteria, are provided.

EXPERIMENTAL

Details of the apparatus used in this investigation have been reported (*12*). The operational principle may be found in the literature (*2, 13, 21, 22*). The "compressed sandwich" packing of the sample (*12*) was found to give highly reproducible results. It was prepared by placing a 150-mg. powdered sample of the polysaccharide between two 200-mg. layers of calcined alumina and compressing at 200 pounds per square inch. The carbohydrates reported in this work were dried over phosphorus pentoxide in order to minimize the effects caused by the water of hydration.

Unless specified otherwise, the thermograms were obtained by heating the sample in an atmosphere of purified nitrogen at the rate of 10° C. per minute. Replicate runs of four to six determinations showed that the endothermal and exothermal temperatures were reproducible to within ±5° in the 25° to 400° C. region and to within ±10° in the 400° to 1000° C. range.

RESULTS AND DISCUSSION

The differential thermographic features of the carbohydrates may be considered to be due primarily to isomeric polymers

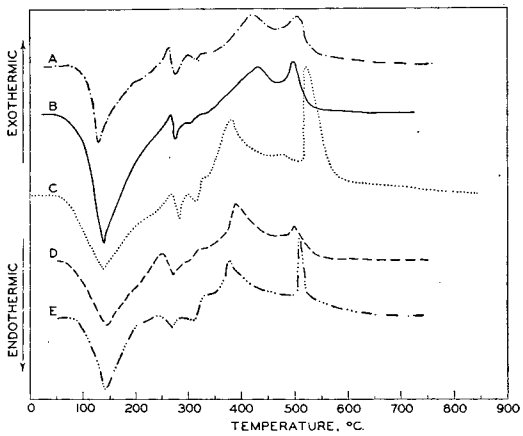

Figure 2. Thermograms of potato and corn starch

A. Potato starch
B. Potato starch duplicate run
C. Corn starch
D. Methanol-extracted corn starch
E. Ammonia-pregelatinized corn starch

composed of glucopyranoside units. The behavior of the two glucose stereoisomers shown in Figure 1 demonstrates the stereospecificity of the thermal reactions. In contrast to α-D-glucose (curve *A*), the β-isomer (curve *C*) displayed a weak but reproducible peak at 170°C. In the 50 to 50 mixture (curve *B*), this peak was detected near 180° C. Similarly, α-D-mannose gave an exothermic peak at 178° C. following its fusion at 142° C.

The differential melting endotherm of α-D-glucose observed at 159° C. (curve *A*) was about 10° C. higher than the accepted melting point. In general, with the rate of heating kept at 10° C. per minute, the recorded melting points were found to be about 10° C. higher than literature values.

The thermograms in Figure 1 show that the monosaccharides usually exhibited an endothermic decomposition soon after melting. For example, in α-D-glucose this appeared at 205° C. A notable exception was the disaccharide cellobiose (*12*).

Starch is considered to be a polymeric glucoside composed of α-1,4- and α-1,6-linked glucopyranosidic units. The thermal properties of these macromolecules are shown in Figure 2. The thermograms of the corn starches graphically illustrate the effect of pretreatment. The samples were provided by the Northern Utilization Research Branch of the United States Department of Agriculture. The potato starch (curves *A* and *B*) had an iodine sorption of 41 mg. per gram. The corn starch (curve *C*), extracted from Iowa 939 corn, was used to prepare the methanol-extracted starch (curve *D*) and the ammonia-pregelatinized starch (curve *E*).

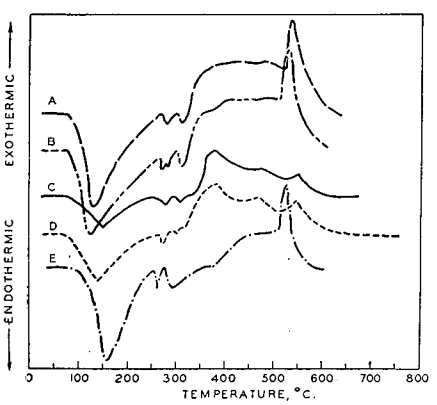

Figure 3. Effect of amylose content on starch thermograms

A. Waxy corn starch from low sulfur dioxide steep
B. Waxy corn starch from high sulfur dioxide steep
C. Acetic acid–extracted wheat starch
D. Acetic acid–extracted wheat starch duplicate run
E. Wrinkled-seeded pea starch

The thermograms of the potato and corn starches were characterized by a procession of endotherms in the 135° to 310° C. region, followed by two distinct exothermic peaks in the 375° to 520° C. range. That the thermal effects were indeed typical of many starch preparations was further exemplified by starches of varying amylose content shown in Figure 3. Curves *A* and *B* represent two samples of waxy corn starch, the former prepared with a low and the latter with a high sulfur dioxide steep. The wrinkled-seeded pea starch contained about 65% amylose according to its iodine sorption. The acetic acid–wheat starch was prepared by the method of Ellington and Purves (*6*).

The thermograms of the linear polymeric fraction of starch—namely, amylose—are depicted in Figure 4. These fractions were prepared from the starch samples shown in Figure 2. The potato amylose (curve *A*), prepared by butyl alcohol fractionation had an iodine sorption of 197 mg. per gram. The ammonia corn amylose (curve *B*) was extracted from the pregelatinized corn starch by butyl alcohol fractionation and recrystallized. It had an iodine sorption of 196 mg. per gram. Examination of the thermograms reveals three prominent features, the endotherms at about 150° and 225° C., a shoulder at 315° C., and a pronounced exothermic peak in the 490° to 510° C. region.

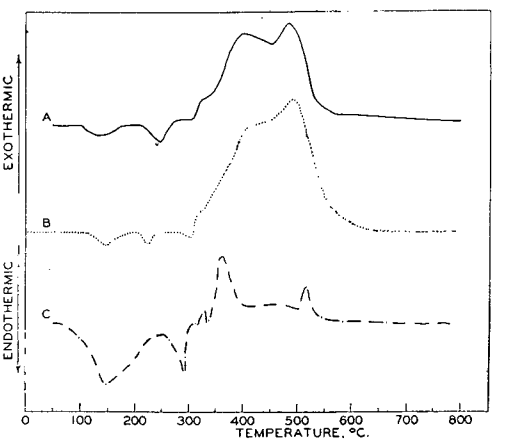

Figure 4. Thermograms of amylose

A. Potato amylose
B. Amylose from ammonia-pregelatinized corn starch
C. Amylose from methanol-extracted corn starch

The branched amylopectin fractions exhibited thermographic contours portrayed in Figure 5. The potato amylopectin (curve *A*) and the amylopectin from ammonia-pregelatinized corn starch were prepared from the starches shown in Figure 2. The wheat (curve *C*) and Easter lily (curves *D* and *E*) amylopectins were supplied by W. Z. Hassid, and their properties have been published elsewhere (*15*). The results showed the similarity of the thermograms with those of the previous amylose fractions. Wheat amylopectin, for example, gave pronounced endotherms at 140° and 275° C., followed by two intense exothermic peaks at 380° and 510° C.

The physicochemical differences between the linear and branched starch fractions have been studied extensively by means of their solution properties. A review of the amylose and

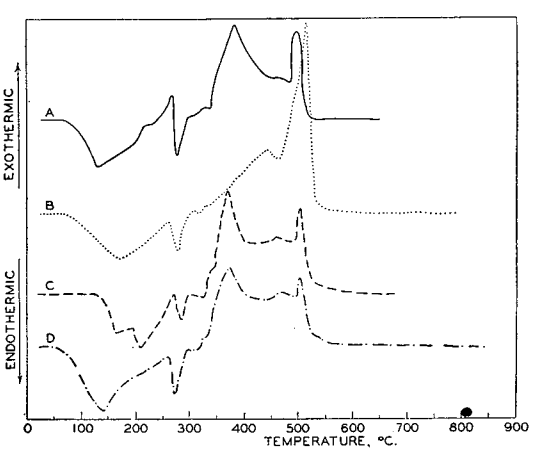

Figure 5. Thermograms of amylopectin

A. Potato amylopectin
B. Amylopectin from ammonia-pregelatinized corn starch
C. Wheat amylopectin
D. Easter lily amylopectin

amylopectin thermograms disclosed that such distinctions based solely upon the differential thermographic features must be made with extreme reservations, for it was not possible to assign a thermal curve which would be representative of either the amylose or the amylopectin fraction. However, a qualified distinction was possible for certain botanical species. This is illustrated by the potato starch fractions in Figure 6, prepared from the same starch (curve *A*). The amylopectin (curve *C*) was characterized by two distinct endotherms at 230° and 270° C., whereas the amylose (curve *B*) exhibited a pronounced endotherm at 295° C. In the 50 to 50 mixture (curve *D*), the components did not behave independently.

There are reasons to believe that starch is intermediate between glycogen and cellulose in certain properties (*18*). Figure 7 illustrates the thermal features of some 1,4- and 1,6-linked glucosidic polymers. Here it was observed that the thermograms of the macromolecules composed of α-linkages—namely, corn starch (curve *A*), D(+) glycogen (curve *C*), and dextran (curve *D*, obtained from *L. mesenteroides* B512, molecular weight 75 × 10^6—were characterized by a procession of endotherms in the 150° to 320° C. region. Cellulose (curve *B*), on the other hand, having a β-1,4-linkage, gave a singularly distinct endotherm at 340° C. Moreover cellulose, in contrast to starch, gave thermograms which were nearly identical regardless of the botanical source of the samples used.

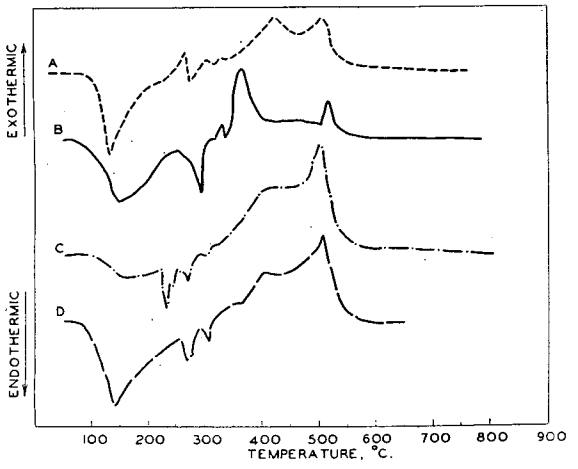

Figure 6. Thermographic identification of starch fractions

A. Potato starch
B. Potato amylose
C. Potato amylopectin
D. 50 to 50 mixture of potato amylose and amylopectin

One may speculate tentatively upon the mechanism of the differential thermal reactions. The nature of thermal degradation of various polysaccharides under selected conditions has been the subject of numerous papers (*3, 14, 17, 20*). In order to ascertain whether the suggested mechanisms may be extrapolated to the conditions of differential thermal analysis, gaseous products evolved during the procedure were examined.

A typical result was shown by wheat starch. Its thermograms (Figure 3, curves *C* and *D*) were characterized by endotherms at 145°, 275°, and 310° C. Qualitative analysis of the gaseous products showed that at 145° and 275° C. water vapor was the predominant pyrolyzate. Neither carbon dioxide nor carbon monoxide was detected. At 310° C. water again was obtained, accompanied by straw-colored gases. The latter were condensed in a cold trap for subsequent analysis.

The data indicated that, when wheat starch was pyrolyzed in admixture with calcined alumina, the following sequence of events may have occurred. The endothermic reactions at 145° and 275° C. involved selective dehydration, possibly accompanied by dextrinization or transglucosidation (*3, 9, 17*). This conjecture is based, in part, on the detection of water as the main pyrolysis product at these temperatures and on the infrared absorption spectra of the pyrolysis residues in Nujol mulls. These spectra showed that, with increasing temperature, there was a gradual decrease in the intensity of the hydroxyl absorption band at 3000 cm.$^{-1}$.

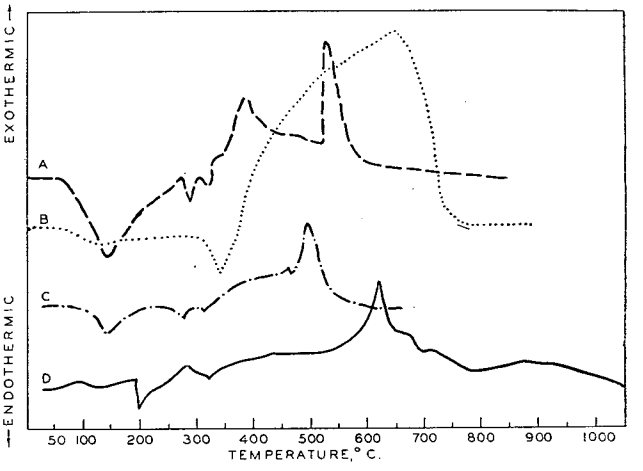

Figure 7. Thermograms of related glucose polymers

A. Corn starch
B. Whatman cellulose powder
C. D(+) glycogen
D. Dextran

The third prominent endotherm at 310° C. appeared to involve further elimination of the polyhydroxyl groups, accompanied by depolymerization and decomposition responsible for the ensuing broad exothermic peak. The experimental basis for this supposition is the observation that a solution of the condensed gaseous product in 95% aqueous ethyl alcohol gave an ultraviolet absorption spectra (ϵ_{max} at 220 and 280 mμ) suggestive of furfural-like substances (*19*). The infrared absorption spectra of the corresponding chloroform and carbon tetrachloride solutions gave prominent absorptions at 2900, 1710, 1460, 1410, 1290, 1110, 1020, and 885 cm.$^{-1}$ This compared favorably with the spectrum of partially polymerized furfural, which showed absorption peaks at 2800, 1700, 1460, 1410, 1290, 1110, 1020, and 885 cm.$^{-1}$ The detection of these compounds under differential thermal conditions was a distinct contrast to the glucosans which have been isolated during vacuum pyrolysis (*5, 7*).

Undoubtedly, the over-all degradation mechanism is a very complex one. Because of the small amounts of volatile products isolated during the course of the analysis, no further attempts were made to detect the oligosaccharides reported in the literature (*5*).

The foregoing results strongly suggest that the alumina cannot be assumed to be completely inert. In fact, thermal analysis of the starches undertaken without the oxide disclosed that, while the endotherms in the 275° C. region remained essentially unaltered, subsequent thermal peaks were either displaced or suppressed. In view of the poor reproducibility of the peaks without the use of alumina, further interpretations did not appear justified. It should be emphasized here that the object in using calcined alumina was twofold—namely, for reproducibility and for the use of small samples. Analyses of 20- to 50-mg. samples are readily accomplished with sandwich packing.

Whatever the true mechanism of the thermal processes may be, the thermograms are manifestations of molecular composition. The differential thermographic contours serve not merely to characterize or identify substances, but eventually to yield important information pertaining to the relation between molecular composition and chemical property.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Astbury, W. T., Bell, F. O., and Hanes, C. S., *Nature* **146**, 558 (1940).
(2) Barshad, I., *Am. Mineralogist* **37**, 667–94 (1952).
(3) Brimhall, B., *Ind. Eng. Chem.* **36**, 72–5 (1944).
(4) Costa, D., and Costa, G., *Chimica e industria (Milan)* **33**, 707 (1951).
(5) Dimler, R. J., *Advances in Carbohydrate Chem.* **7**, 37–52 (1952).
(6) Ellington, A. C., and Purves, C. B., *Can. J. Chem.* **31**, 801–13 (1953).
(7) Hann, R. H., and Hudson, C. S., *J. Am. Chem. Soc.* **64**, 2435–8 (1942).
(8) Hellman, N. N., Boesch, T. F., and Melvin, E. H., *Ibid.* **74**, 348–50 (1952).
(9) Kerr, R. W., and Cleveland, F. C., *Die Stärke* **5**, 261–6 (1953).
(10) Kerr, R. W., Cleveland, F. C., and Katzbeck, W. J., *J. Am. Chem. Soc.* **73**, 11–17 (1951).
(11) Lansky, S., Koai, M., and Schoch, T. J., *Ibid.*, **71**, 4066–75 (1949).
(12) Morita, H., and Rice, H. M., ANAL. CHEM. **27**, 336–9 (1955).
(13) Penther, C. J., Abrams, S. T., and Stross, F. H., *Ibid.* **23**, 1459–66 (1951).
(14) Picon, M., *Bull. schim. France* **1953**, 681–6.
(15) Potter, A. L., and Hassid. W. Z., *J. Am. Chem. Soc.* **70**, 3774–7 (1948).
(16) *Ibid.*, **73**, 593–5 (1951).
(17) Stacey, M., and Pautard, F. G., *Chemistry & Industry* **1952**, 1058–9.
(18) Staudinger, H., *Ann. Chem. Justus Liebigs* **530**, 1 (1937).
(19) Turner, J. H., Rebers, P. A., Barrick, P. L., and Cotton, R. H., ANAL. CHEM. **26**, 898–901 (1954).
(20) Ulmann, M., *Kolloid-Z.* **130**, 31–9 (1953).
(21) Vold, M. J., ANAL. CHEM., **21**, 683–8 (1949).
(22) Whitehead, W. L., and Breger, I. A., *Science* **111**, 279–81 (1950).
(23) Wolff, I. A., Gundrum, L. J., and Rist, C. E., *J. Am. Chem. Soc.* **72**, 5188–91 (1950).

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Thermometric Titration of Stannic Chloride

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Thermometric titration is presented as a method for the determination of Lewis acids in organic solvents. Stannic chloride can be titrated with dioxane in benzene or carbon tetrachloride with an accuracy of within about 1% and in nitrobenzene or chloroform with lesser accuracy. Calorimetric measurements have been used to explain the effect of solvent on the titration, and the useful concentration ranges for these titrations have been presented.

NUMEROUS reports on the use of thermometric titrations for aqueous solutions may be found in the literature. Linde, Rogers, and Hume (*3*) have presented a comprehensive list of references on the subject, together with a discussion of the principles involved and an indication of the general applicability of the method. In contrast, very few applications to nonaqueous systems have been made (*6*, *7*). This fact is surprising in view of the scarcity of end-point detection methods: Neither electrometric techniques nor indicators are generally applicable to nonaqueous solutions. Furthermore the lower specific heats of many organic solvents, as compared with water, introduce a favorable sensitivity factor. These considerations suggested the applicability of thermometric titrations for the determination of Lewis acids in connection with the authors' studies of acid-base reactions in aprotic solvents. The successful titration of aluminum chloride by this method has been reported (*7*), although experimental details regarding the concentration range and effect of solvent are lacking.

Rice, Zuffanti, and Luder (*5*) have investigated, qualitatively, the behavior of indicators in solutions of Lewis acids and bases, but their general suitability has not been demonstrated quantitatively. Conductometric titrations, as used by La Mer and Downes (*2*) for Brönsted acids in benzene, gave unsatisfactory results.

Stannic chloride was chosen for this investigation because of its solubility in the aprotic solvents employed. Thus the reactions are homogeneous, in contrast with those involving aluminum chloride. Dioxane was selected as the base because its stoichiometry appeared simpler than those of some other bases studied. Four common solvents were used: benzene, carbon tetrachloride, chloroform, and nitrobenzene. The stoichiometry was studied as a function of acid and base concentrations, and the effect of the solvent was investigated. Calorimetric measurements were made in order to explain the results and indicate how these data can be used to indicate the suitability of solvents.

EXPERIMENTAL

Apparatus. The uniform feed buret was similar to Lingane's (*4*), and the temperature measurement and recording system for thermometric titration was like that of Linde, Rogers, and Hume (*3*). A Western Electric 14A thermistor may be used where greater sensitivity is desirable, but it requires the use of a high-input-impedance recorder.

For calorimetric measurements, the thermistor was calibrated against a standard thermometer using a Wheatstone bridge to measure resistance. The calibration was performed at several points over the narrow working temperature range, and resistances were converted to temperatures by means of standard procedures (*1*).

Reagents. STANNIC CHLORIDE, anhydrous, Baker's analyzed reagent.

DIOXANE, technical grade, was refluxed with hydrochloric acid, neutralized with potassium hydroxide, dried over calcium chloride, then refluxed and distilled over sodium.

BENZENE, thiophene free, was shaken with sulfuric acid, washed with water, dried over calcium chloride, then refluxed and distilled over sodium.

CARBON TETRACHLORIDE, technical grade, was refluxed and distilled over calcium chloride.

CHLOROFORM, technical grade, was refluxed and distilled over calcium chloride.

NITROBENZENE, reagent grade, was distilled under vacuum.

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Procedure. For thermometric titrations, concentrated solutions of stannic chloride in the appropriate solvents were prepared immediately before use. These solutions were stable with respect to atmospheric moisture, in contrast with pure stannic chloride. This fact was verified experimentally; nevertheless precautions were taken to exclude moisture. Portions of these solutions were delivered by means of a weight buret to 80-ml. samples of solvent, contained in the titration vessel. When constant temperature was attained, these solutions were titrated with a solution of dioxane in the same solvent. Since solution preparation and titration were performed at the same temperature, no volumetric errors could result from changes in solution density. Concentrations of dioxane were chosen so that all titrations required between 6 and 8 ml. of titrant, which was delivered with an accuracy of within 0.3%.

Calorimetric measurements were made by combining the various liquids, after permitting them, and the Dewar flask, to reach ambient temperature. For the measurement of ΔH_2 and ΔH_4, the integral heats of dilution of pure stannic chloride and of pure dioxane, respectively, the acid and base, respectively, were added from a calibrated pipet to 100 ml. of the solvent. To obtain ΔH_3, the heat of reaction between stannic chloride solution and pure dioxane, the base was added in slight excess of the stoichiometric amount to the resultant solution from the determination of ΔH_2. Temperature-time curves were plotted and temperature differences were read within an accuracy of 0.01° C. Calculations of ΔH were based on the heat capacity of the solvent, rather than that of the solution. This leads to but a small error since the solutions are dilute, approximately 0.1*M*. The heat capacity of the calorimeter itself was neglected, although this also leads to an error, which may approach 0.2 kcal. per mole (*8*). These errors, together with that involved in the addition of one liquid to the other, limit the over-all accuracy to about 0.5 kcal. per mole.

RESULTS AND DISCUSSION

All titration curves show only one end-point break regardless of the solvent used, and their appearance is conventional (*3*). The quantity of heat evolved during the titration depends upon the solvent used. It increases in the following order: chloroform < nitrobenzene < benzene, carbon tetrachloride. In all solvents, except chloroform, a dense white precipitate appears during the titration as the reaction product. The results are summarized in Table I.

Table I. Stoichiometry of Stannic Chloride–Dioxane Titrations

Solvent	Benzene	Carbon Tetrachloride	Nitrobenzene	Chloroform
$SnCl_4$ concn. range[a]	3–15	3–35	6–24	6–90[b]
Mean molar ratio[c]	0.99	1.01	1.04	0.96
% std. dev.	1.4	0.8	5.0	1.3
No. of detns.	17	6	7	5

[a] Values indicated are millimoles of $SnCl_4$ per 100 ml. solvent. These represent the useful range.
[b] No upper limit was reached, hence this represents only the highest value investigated.
[c] This ratio is calculated from the end-point volumes and solution concentrations.

The concentration ranges indicated are those in which satisfactory titrations may be performed within the limits of accuracy shown. At the upper end, the dense white precipitate, which coats the thermistor, prevents rapid heat transfer and places a limit on the accuracy of the end-point extrapolation in the cases of carbon tetrachloride, benzene, and nitrobenzene. The lower limit is determined by the required sharpness of the end-point break, which is a function of the rate of heat evolution. Thus, the optimum concentrations would be those closest to the upper limits shown in Table I.

In benzene or carbon tetrachloride, stannic chloride can be titrated with an accuracy of within 1% within the concentration ranges indicated, and in nitrobenzene accuracy within 5% can be attained. Chloroform appears to be a poor solvent for this titration because of the very small heat evolution, $-\Delta H_3$ in Table II. The low per cent standard deviation in the latter case is fortuitous, as the end-point break is not sharp and the extrapolation is inaccurate. Both the titration curves and the calorimetric observations indicate that the reaction in chloroform is more complicated than those which take place in other solvents. A slow reaction seems to be superimposed on the more rapid one. This slow reaction appears to be the crystallization of the product, since on standing a precipitate gradually appears. The same experimental results are observed when using very high purity chloroform, so that the difference in behavior between chloroform and the other solvents is not attributable to the presence of impurities.

Table II. Calorimetric Data

Temperature, 22.0 ± 1.0° C.[a]

Solvent	Benzene	Carbon Tetrachloride	Nitrobenzene	Chloroform
$-\Delta H_1$[b]	16.2	15.9	19.9	24.5
$-\Delta H_2$[c]	−0.5	0.1	6.7	18.9
$-\Delta H_3$[d]	16.7	15.8	13.2	5.6
$-\Delta H_4$[e,f]	0.2	0.1	0.4	2.2
Dielectric constant	2.3	2.2	35	4.5
Dipole moment	0	0	4.2	1.1

[a] Mean temperature over which heat data is taken. Variation, ±1.0° C., corresponds to maximum variation between different runs. During any one run, it is constant to within ±0.01° C.
[b] $-\Delta H_1 = -\Delta H_3 - \Delta H_2$. It is calculated from the measured values of $-\Delta H_3$ and $-\Delta H_2$. All ΔH values are given in kcal.
[c] $-\Delta H_2$ is the measured integral heat of dilution of pure $SnCl_4$ per mole $SnCl_4$. The final concentration is 8.5×10^{-2} mole per liter at 22.0 ± 1.0° C.
[d] $-\Delta H_3$ is the measured heat of reaction, per mole of $SnCl_4$, of pure dioxane with the solution of $SnCl_4$ used to obtain $-\Delta H_2$. Dioxane is added in very slight excess of molar ratio of unity.
[e] $-\Delta H_4$ is the measured integral heat of dilution of pure dioxane, per mole of dioxane. The final concentration is 8.5×10^{-2} mole per liter at 22.0 ± 1.0° C.
[f] All measured values are the mean of at least two determinations with a mean deviation no greater than 0.5 kcal. per mole.

The factors which govern the quantity of heat evolved during the titration may be explained in terms of the calorimetric data which are presented in Table II. Generally, the titration reaction may be written:

$$\text{Stannic chloride (solution)} + \text{dioxane (solution)} = \text{product} + \text{solvent} - \Delta H_5 \quad (1)$$

where $-\Delta H_5$ represents the heat evolved per mole of stannic chloride titrated. This may be analyzed in terms of the following reactions:

$$\text{Stannic chloride} + \text{solvent} = \text{stannic chloride (solution)} - \Delta H_2 \quad (2)$$

$$\text{Stannic chloride (solution)} + \text{dioxane} = \text{product} + \text{solvent} - \Delta H_3 \quad (3)$$

$$\text{Dioxane} + \text{solvent} = \text{dioxane (solution)} - \Delta H_4 \quad (4)$$

and by adding Equations 2 and 3, Equation 5 is obtained.

$$\text{Stannic chloride} + \text{dioxane} = \text{product} - \Delta H_1 \quad (5)$$

Thus $-\Delta H_1$, which is the sum of $-\Delta H_2$ and $-\Delta H_3$, should be the same in all solvents if the resultant product involves dioxane and stannic chloride alone. It may be seen from the data in Table II that for benzene and carbon tetrachloride this is true within the limits of experimental error; on the other hand, nitrobenzene and chloroform show large differences from this value. This fact cannot be explained in terms of solvent polarity alone, as may also be seen from Table II.

The measured value of $-\Delta H_3$ is very nearly equal to that of $-\Delta H_5$; it is larger than $-\Delta H_5$ by about 20% of $-\Delta H_4$, which is almost negligible. The difference between $-\Delta H_3$ and $-\Delta H_5$ is that quantity of heat liberated when dioxane is dissolved in the solvent to form the titrant solution. Examination of the data

shows that whereas $-\Delta H_1$ and $-\Delta H_2$ both increase in the order: benzene, carbon tetrachloride < nitrobenzene < chloroform, the change in $-\Delta H_2$ is much greater than that in $-\Delta H_1$, with the result that $-\Delta H_3$ increases in the reverse order. In choosing a solvent, therefore, consideration must be given to the heat of interaction between Lewis acid and solvent, $-\Delta H_2$, as well as to the heat of interaction between base and solvent, $-\Delta H_4$; the greater the values of $-\Delta H_2$ and $-\Delta H_4$, the smaller will be the heat developed during the titration. For the bases used in this investigation $-\Delta H_4$ was negligible, although it may not be so for other bases.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Becker, J. A., Green, C. B., and Pearson, G. L., *Bell System Tech. J.* **26**, 170 (1947).
(2) La Mer, V. K., and Downes, H. C., *J. Am. Chem. Soc.* **53**, 888 (1931).
(3) Linde, H. W., Rogers, L. B., and Hume, D. N., ANAL. CHEM. **25**, 404 (1953).
(4) Lingane, J. J., *Ibid.*, **20**, 285 (1948).
(5) Rice, R. V., Zuffanti, S., and Luder, W. F., *Ibid.*, **24**, 1022 (1952).
(6) Somiya, T., *J. Soc. Chem. Ind., Japan* **51**, 135T (1932).
(7) Trambouze, Y., *Compt. rend.* **233**, 648 (1951).
(8) Vold, R. D., *J. Am. Chem. Soc.* **59**, 1515 (1937).

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Determination of Aldehydes by Mercurimetric Oxidation

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A method of analysis has been developed for the determination of aldehydes, which is based on the oxidation of aldehyde to acid by mercuric ion which, in turn, is reduced to free mercury. The analysis is concluded by an iodometric measurement of the mercury. The method is applicable to the determination of virtually any concentration of aldehyde in the presence of most alcohols, acids, esters, acetals, ketones, ethers, organic chlorides, and epoxides. Reaction conditions and purity data are presented for 12 aldehydes which can be determined by the method.

A COMMON problem in organic analysis is the quantitative analytical resolution of mixtures containing both the aldehyde and ketone functions. Various hydroxylamine hydrochloride procedures have been developed to determine the total carbonyl value of such a mixture, but they are not specific for aldehydes. Several methods have been proposed for the determination of aldehydes by oxidation with silver compounds, such as the procedures of Mitchell and Smith (*10*) and Siggia (*11*). An excellent review of the field of carbonyl analysis has been prepared by Mitchell (*9*).

Previous investigators who have attempted the determination of aldehydes by some mercurimetric procedure have recommended the method primarily for the estimation of formaldehyde (*1, 2, 12*). In addition, Bougault and Gros (*3*) have reported the determination of furfural, benzaldehyde, and piperonal, and Goswami, Das-Gupta, and Ray (*5*), Goswami and Das-Purkaystha (*6*), and Goswami and Shaha (*7*) have estimated sugars with various degrees of success using empirical factors.

These investigators all employed an alkaline solution of potassium mercuric iodide, K_2HgI_4, as an oxidizing agent. In the reaction, aldehyde is oxidized to the corresponding acid whereas mercuric ion is reduced to free mercury:

$$RCHO + K_2HgI_4 + 2KOH \longrightarrow RCOOH + Hg^\circ + 4KI + H_2O$$

Both isolation and nonisolation methods have been proposed for the determination of the free mercury. In the authors' opinion it is best to acidify the reaction mixture and react the free mercury with a measured excess of iodine. The amount of iodine consumed is a stoichiometric function of the free mercury which, in turn, is a measure of the aldehyde originally present. Agar is employed as a protective colloid to maintain the free mercury in a finely divided state, thus promoting its reaction with iodine.

The name, "mercural reagent," has been coined to differentiate the reagent from other potassium mercuric iodide preparations such as Nessler's reagent. "Mercural" signifies a mercuric oxidation of aldehydes.

REAGENTS

Mercural Reagent. To 1830 ml. of distilled water contained in a 1-gallon jug add 150 grams of reagent grade potassium chloride, 240 grams of U. S. Pharmacopeia grade mercuric chloride (mercury bichloride), 642 grams of reagent grade potassium iodide, and 1000 ml. of an aqueous 40% by weight potassium hydroxide solution. Shake the contents after each addition to ensure complete solution. This reagent is stable and does not deteriorate on standing. The slight amount of yellow or brown precipitate which may form is assumed to be due to ammonium ion in the reagents, however, it is not detrimental to the effectiveness of the reagent.

Agar Solution, 0.1%. Add 3.0 grams of Difco Bacto-Agar to 300 ml. of boiling distilled water. Continue heating with occasional swirling until the solid has dissolved and the resulting solution is essentially clear. Cool and dilute to 3 liters with additional distilled water. Add 0.1 gram of mercuric iodide as a preservative and shake vigorously for a few seconds.

Acetic Acid, analytical reagent grade

Iodine, approximately 0.1*N*

Starch Indicator, 1.0% solution

Standard 0.1*N* Sodium Thiosulfate

Methanol, commercial grade, Carbide and Carbon Chemicals Co.

SAMPLING

Unless direct sample addition is specified, introduce the sample into a tared 50-ml. volumetric flask containing 30 ml. of the required solvent (methanol which has been neutralized to bromothymol blue indicator, or distilled water) using a hypodermic syringe fitted with a 3-inch needle and chilled if necessary to facilitate transfer. Stopper the flask and swirl to effect solution. An acetaldehyde dilution must be allowed to stand for approximately 15 minutes, with occasional venting to the atmosphere to reach equilibrium before recording the gross weight. The gross weight of dilutions of other aldehydes may be determined immediately. Dilute to the mark with additional solvent and mix thoroughly. A 5-ml. aliquot of this dilution should contain not more than 3.0 meq. of aldehyde. Fill the pipet by pressure to avoid loss of aldehyde.

If the sample is weighed directly into the reagent, care must

be exercised to shake the flask vigorously at once to intimately mix the contents and prevent localized side reactions.

PROCEDURE

The determination is best performed in 500-ml., Erlenmeyer glass-stoppered flasks which are fitted with 24/40 ground-glass joints. Prepare sample and blank flasks by adding 50 ml. of mercural reagent to each. Consult Table I for the proper reaction temperature and, if necessary, cool each of the flasks in a wet-ice bath for 10 minutes. With constant swirling during the addition, introduce an amount of sample containing not more than 3.0 meq. of aldehyde using the procedure specified in Table I. If a dilution is used, add a similar amount of solvent to the blank. Allow the flasks to stand together at the temperature and for the length of time specified in Table I. Add 50 ml. of agar solution to each flask and swirl vigorously for approximately 1 minute to disperse the mercury precipitate, then add 25 ml. of glacial acetic acid with constant agitation during the addition.

Table I. Sampling Procedure and Reaction Conditions for Determination of Aldehydes by Mercural Procedure

Compound	Maximum Sample Size for Pure Material, G.[a]	Reaction Time, Min.[b]
Acetaldehyde	0.66	5 to 60
Acetaldol	1.3	5 to 60[e]
Acrolein	0.84[c]	180 to 240[d]
Benzaldehyde	1.6[c]	15 to 60[d]
Butyraldehyde	1.1	30 to 60
2-Ethylbutyraldehyde	0.15[e]	15 to 60[f]
Formaldehyde	0.45	1 to 60
Glutaraldehyde	0.75	15 to 60
Hexaldehyde	0.15[e]	30 to 60[f]
Isobutyraldehyde	1.1[c]	5 to 60[d]
Methacrolein	0.90[c]	15 to 60[d]
Propionaldehyde	0.87	15 to 60

[a] Use distilled water as a solvent in the sample dilution unless otherwise specified.
[b] Minutes at room temperature unless otherwise specified.
[c] Use methanol, which has been neutralized to bromothymol blue indicator, as the dilution solvent.
[d] Minutes in a wet-ice bath (0° to 3° C.).
[e] Add the sample directly to the sample flask, stopper, and immediately shake the contents vigorously by hand for 1 minute prior to the mechanical shaking.
[f] Minutes on a mechanical shaker.

If the sample contains acetaldehyde, allow the flasks to stand at room temperature for approximately 15 minutes before proceeding. The standing period is not required for samples of other aldehydes. Pipet exactly 50 ml. of approximately 0.1*N* iodine into each flask, using pressure to fill the pipet. Stopper each flask and shake vigorously until all of the gray mercury precipitate goes into solution. If necessary, place on a mechanical shaker for 5 minutes. Carefully remove each stopper, rinse any adhering liquid into the flask, and rinse down the inside walls of the flask with distilled water. Titrate with standard 0.1*N* sodium thiosulfate until the brown iodine color begins to fade. Add a few milliliters of starch indicator solution and continue the titration just to the disappearance of the blue color, approaching the end point dropwise while swirling constantly. From the difference between blank and sample titrations the percentage of aldehyde present in the sample can be calculated; one aldehyde group consumes two equivalents of iodine:

$$-CHO \equiv Hg^{\circ} \equiv I_2 \equiv S_2O_3^{--}$$

Hence for monoaldehydes the equivalent weight is one half of the molecular weight.

DISCUSSION

The reagent originally investigated was of the composition usually specified as Nessler's reagent, although, generally speaking, no two authors use the same formulation. However, it was found at this point that Nessler's reagent would not quantitatively oxidize most aldehydes.

A study of the reagent was therefore initiated to determine its optimum composition. Experiments were conducted to determine the effect of the following variables: concentration of potassium mercuric iodide complex, concentration of potassium hydroxide, and ratio of potassium iodide to mercuric chloride. In each case a sample of acetaldehyde was reacted for 1 hour at room temperature with approximately 50 ml. (70 grams) of reagent. Results showed 10 to 20% by weight of the potassium mercuric iodide complex in solution gave quantitative results. Likewise, a potassium hydroxide content of 10 to 20% by weight afforded a quantitative oxidation of acetaldehyde. Higher percentages of either component caused solubility difficulties, whereas lesser amounts resulted in incomplete reaction. Variation of the potassium iodide–mercuric chloride ratio indicates best results were obtained when the ratio of iodide to mercuric ions was slightly higher than the 4 to 1 of the potassium mercuric iodide, K_2HgI_4, complex. Any ratio less than 4 to 1 tended to produce an undesirable precipitate of mercuric iodide, whereas a ratio significantly higher than 5 to 1 not only gave low results, but also impaired the effectiveness of the agar used as a protective colloid, yielding a mercury precipitate which was less reactive with iodine.

On the basis of these experiments a reagent was formulated to contain 16% by weight potassium mercuric iodide, 13% by weight potassium hydroxide, and approximately 1 gram of excess potassium iodide per 50 ml. of reagent.

Using this reagent, experiments were undertaken to establish the necessary reaction conditions for pure aldehydes. Water-soluble aldehydes were sampled in the form of aqueous dilutions and oxidized at room temperature. As a mutual solvent for higher molecular weight aldehydes, methanol has proved satisfactory. Its exact use depends on the particular aldehyde, but the usual procedure is to employ neutralized methanol as a dilution solvent and conduct the reaction at the temperature of a wet-ice bath (0° to 3° C.) to prevent any oxidation of the methanol. In some instances direct addition of sample to reagent, accompanied by shaking, is the best procedure. The most suitable method of sampling, reaction conditions, and sample size for a number of aldehydes for which this procedure has been found satisfactory, are given in Table I.

RESULTS

Comparable data on the purity of a number of aldehydes were obtained by the mercural procedure and a hydroxylamine hydrochloride–triethanolamine method (*4*). The average result, the precision attained, and the number of determinations for each sample are given in Table II.

Table II. Purity Determinations on Aldehydes by Mercural and Hydroxylamine Procedures

Compound	Purity by Mercural Procedure[a], %	Purity by Hydroxylamine Procedure[b], %
Acetaldehyde	98.9 ± 0.3 (5)	98.9 ± 0.3 (4)
Acetaldol	101.5 ± 0.2 (2)	101.6 ± 0.3 (3)
Acrolein	98.8 ± 0.3 (5)	99.0 ± 0.0 (2)
Benzaldehyde	95.3 ± 0.2 (8)	95.3 ± 0.2 (5)
Butyraldehyde	98.0 ± 0.5 (11)	97.7 ± 0.5 (7)
2-Ethylbutyraldehyde	96.5 ± 0.3 (3)	96.9 ± 0.1 (2)
Formaldehyde	35.9 ± 0.1 (6)	35.7 ± 0.1 (2)
Glutaraldehyde	26.3 ± 0.05 (4)	.....
Hexaldehyde	95.4 ± 0.2 (4)	94.7 ± 0.3 (2)
Isobutyraldehyde	97.7 ± 0.3 (9)	97.4 ± 0.1 (2)
Methacrolein	90.7 ± 0.1 (3)	90.6 ± 0.1 (2)
Propionaldehyde	97.1 ± 0.0 (4)	96.8 ± 0.4 (5)

[a] Figures in parentheses represent number of determinations.
[b] Hydroxylamine hydrochloride–triethanolamine (*4*).

Sufficient purity determinations for statistical treatment of data were conducted on a given sample of acetaldehyde by the mercural method. The standard deviation for the determination of acetaldehyde using aqueous dilutions was 0.39% for 13 degrees of freedom on a sample whose average purity was 97.5%. The sampling error was not significant.

The procedure has been modified and found suitable for the determination of trace amounts of aldehydes in organic compounds. For example, determinations of acetaldehyde in ethylene oxide and propionaldehyde in propylene oxide have been performed successfully. The method used is similar to the one previously described.

Transfer 25 ml. of mercural reagent to each flask, add 25 ml. of distilled water, and chill the flasks to 0° to 3° C. in a wet-ice bath. Add 20 ml. of the chilled epoxide sample from a graduate, swirl the flasks, and return to the ice bath for 60 minutes. Add 50 ml. of agar solution and 150 ml. of distilled water to each flask and swirl vigorously. Pipet exactly 25 ml. of approximately 0.1*N* iodine into each flask and swirl until the gray mercury precipitate has completely reacted. Titrate the excess iodine with standard 0.1*N* sodium thiosulfate until the brown color begins to fade. Add a few milliliters of starch indicator solution and continue to titrate to the disappearance of the blue color.

A synthetic sample prepared to contain 0.037% propionaldehyde in propylene oxide analyzed 0.036% by this procedure, and a synthetic containing 0.064% acetaldehyde in ethylene oxide gave a result of 0.061%. In addition, comparative analyses of acetaldehyde in ethylene oxide were performed on two synthetic samples by the mercural procedure and a sodium bisulfite–iodine method (*4*). One sample contained 10 ± 4 p.p.m. acetaldehyde by the mercural procedure, whereas the sodium bisulfite method gave 5 ± 4 p.p.m. A second sample was 61 ± 6 p.p.m. acetaldehyde by the mercural procedure and 60 ± 10 p.p.m. using bisulfite.

INTERFERENCE STUDIES

Many organic compounds do not interfere with this procedure, permitting the determination of aldehyde in the presence of most acids, ketones, esters, acetals, ethers, alcohols, epoxides, and organic chlorides.

Oxidation studies were conducted on methanol, ethyl alcohol, isopropyl alcohol, and butyl alcohol, both at room temperature and at the wet-ice bath temperature. Methanol is slowly attacked by the reagent at room temperature, but is completely resistant to oxidation at 0° to 3° C. and is, therefore, a preferred nonaqueous solvent for some aldehydes, as indicated in Table I. Isopropyl alcohol is the worst offender, not only because it is oxidized even at 0° to 3° C., but also because its oxidation product, acetone, complexes the mercuric ion. Ethyl and butyl alcohols are only slightly oxidized at 0° to 3° C. Studies indicate that the oxidation of alcohols by mercural reagent follows the mass action law, enabling one to compensate for this deleterious reaction by using a reagent diluted 50 to 50 with distilled water, adding a similar amount of alcohol to the blank, and performing the oxidation in a wet-ice bath, allowing a suitably longer reaction time to offset the dilution of reagent and reduction in temperature. Errors introduced by this procedure are not serious when alcoholic samples containing only a few per cent aldehyde are involved. Samples containing esters require the same conditions, because they are saponified to alcohols by the potassium hydroxide in the reagent.

Some vinyl compounds are known to interfere with this procedure by adding iodine, thus yielding a high result; in the case of vinyl ethers, the addition is essentially quantitative. This method has been found applicable to the determination of acrolein (acrylaldehyde) and methacrolein (methacrylaldehyde) (see Table II), whereas crotonaldehyde has been analyzed with an accuracy of within ±2%. However, no satisfactory results have been obtained on unsaturated aldehydes containing more than four carbon atoms—e.g., 2,4-hexadienal (sorbaldehyde), 2-ethylcrotonaldehyde, and 2-ethyl-3-propylacrolein. Therefore, the determination of unsaturated aldehydes or of aldehyde in any mixture containing an unsaturated compound must be checked for interference.

Acetone reacts with mercuric ion in the following manner (*8*):

$$Hg^{++} + 2CH_3{-}\overset{\overset{O}{\|}}{C}{-}CH_3 \rightleftharpoons Hg(CH_3{-}\overset{\overset{O}{|}}{C}{=}CH_2)_2 + 2H^+$$

In the presence of the alkaline reagent and excess mercuric ions this equilibrium reaction is displaced to the right, depositing the mercuric ion–acetone complex as a yellow solid. On acidification the reaction is reversed, proceeding to the left. This reversal must be complete, as indicated by the absence of the yellow precipitate, or else iodine is consumed, presumably through iodination of the double bonds.

Lower temperatures induce precipitation or even resinification of the mercuric ion–acetone complex, hence greater solubility difficulties are experienced at 0° to 3° C. than at room temperature.

In order to illustrate the effect of the presence of acetone, a series of blank determinations was made as specified in the method, using reaction conditions of 30 minutes at 0° to 3° C. From 0 to 3.0 grams of acetone were added to each flask. With the addition of up to 0.3 gram of acetone, a yellow precipitate was formed which easily dissolved on acidification. More than 0.3 gram of acetone caused deposition of a resin, requiring additional potassium iodide to effect solution. Hence, the acetone tolerance of this method is approximately 0.3 gram for determinations performed in a wet-ice bath.

Because a portion of the mercuric ion contained in 50 ml. of reagent is complexed by 0.3 gram of acetone, it was then necessary to prove that a sufficient amount of reagent was still available for the quantitative determination of aldehyde. Results on the determination of propionaldehyde in the presence of 0.3 gram of acetone show quantitative oxidation is attained even when the maximum sample size of propionaldehyde is taken.

Methyl ethyl ketone complexes mercuric ion to a much smaller degree than acetone, whereas methyl isopropyl ketone and ethyl butyl ketone are practically inert.

Hydroxy ketones constitute a positive interference, as do other easily oxidized substances or anything which consumes iodine. Conversely, oxidizing agents such as peroxides are likely to produce low results, either by competing with mercuric ion in the oxidation of aldehyde or by oxidizing iodide to iodine.

As a rule the amount of acid or ester which can be tolerated must not be so great as to neutralize more than one third of the potassium hydroxide in the reagent, whereas no more than one half of the mercuric ion content should be reduced and/or complexed.

LITERATURE CITED

(1) Alexander, E. R., and Underhill, E. J., *J. Am. Chem. Soc.* **71**, 4014–19 (1949).
(2) Bolle, J., Jean, J., and Jullig, T., *Mém. services chim. état (Paris)* **34**, 317–20 (1948).
(3) Bougault, J., and Gros, R., *J. pharm. chim.* **26**, 5–11 (1922).
(4) Carbide and Carbon Chemicals Co., South Charleston, W. Va., unpublished method.
(5) Goswami, M., Das-Gupta, H. N., and Ray, K. L., *J. Indian Chem. Soc.* **12**, 714–18 (1935).
(6) Goswami, M., and Das-Purkaystha, B. C., *Ibid.*, 13, 315–22 (1936).
(7) Goswami, M., and Shaha, A., *Ibid.*, 14, 208–13 (1937).
(8) Fernandez, J. B., Snider, L. T., and Rietz, E. G., ANAL. CHEM. **23**, 899–900 (1951).
(9) Mitchell, J., Jr., "Determination of Carbonyl Compounds" in "Organic Analysis," vol. 1, p. 243, Interscience. New York, 1953.
(10) Mitchell, J., Jr., and Smith, D. M., ANAL. CHEM. 22, 746–50 (1950).
(11) Siggia, S., "Quantitative Organic Analysis via Functional Groups," 2nd ed., pp. 32–6, Wiley, New York, 1954.
(12) Stüve, W., *Arch. Pharm.* **244**, 540 (1906).

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Determination of Benzoic Acid in Alkyd Resins

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The use of benzoic acid in alkyd resin manufacture is increasing. It is particularly useful to control the viscosity of alkyd resins made with isophthalic acid. Its effect on the amount of polyhydric alcohols and dicarboxylic acids needed has increased its value analytically. Benzoic acid can be measured in alkyd resins by ultraviolet spectroscopy after removal of the dicarboxylic and fatty acids. This analytical technique provides a method for its control for qualification purposes.

BENZOIC acid has been used in limited amounts for a number of years in the manufacture of alkyd resins for special purposes. Recently, it has been used in alkyd resins made with isophthalic acid to control the viscosity. Analytically, its value has increased because of its effect on the amount of polyhydric alcohols and dicarboxylic acids needed. A study has been made to determine the effect of benzoic acid on existing methods of analysis for alkyd resins in general, and to obtain a quantitative measure of the acid.

Because of the solubility of its potassium salt in the anhydrous alcohol from which the salts of the other acids precipitate, benzoic acid offers no interference in the usual analysis for dicarboxylic acids (*1*). The potassium benzoate formed passes into the filtrate with the polyhydric alcohols and fatty acid soaps. When the solvents are evaporated from this filtrate and the aqueous solution acidified to release fatty acids, benzoic acid is also formed and is extracted along with the fatty acids by the usual ethyl ether extraction. The benzoic acid cannot be washed entirely from the ether layer with water; thus, it interferes with the usual determination of fatty acids. In order to obtain satisfactory analysis of either the fatty acids or the benzoic acid, a quantitative separation is necessary. Efforts to effect this separation consisted of testing various water-insoluble solvents suitable for extracting fatty acids in which benzoic acid has limited solubility. Such solvents as benzene, chloroform, naphtha, and carbon tetrachloride have been tested, the best results being obtained with the carbon tetrachloride. Although benzoic acid has shown some solubility in all solvents tested, its solubility in carbon tetrachloride is sufficiently low to permit its removal with water washing.

The combined water layer and washings from the fatty acid separation contain benzoic acid, polyhydric alcohols, and large quantities of potassium chloride. Of these, only benzoic acid should show appreciable absorption in the ultraviolet region when spectrophotometric measurement is applied. As shown in Figure 1, this acid shows some absorption throughout most of the ultraviolet region with a well defined peak of maximum absorption at the 273 mμ wave length. The absorptivity of benzoic acid that has been carried through the entire procedure, as described here, is slightly higher than its absorptivity determined directly. This difference can be corrected either by standardizing with benzoic acid treated through all steps of resin analysis, or by using pure benzoic acid as standard and applying a correction factor to all analyses. The latter plan was found preferable, because the former requires the running of a blank which is time-consuming and slightly less accurate. In addition, all alkyd resins tested, which theoretically contain no benzoic acid, show a little absorption at 273 mμ which varies proportionally with the original resin sample weight, but is independent of the type of polyhydric alcohol present. The empirical correction factor (0.0097), applied in all calculations, corrects also for this absorption. A Beckman spectrophotometer, Model DU, was used with 1-cm. cells.

PROCEDURES

Calibration. To determine the absorptivity of benzoic acid, 50 mg. of the acid of highest purity are carefully weighed and dissolved in absolute methanol and diluted to 100-ml. volume. To a 10-ml. aliquot of this methanol solution are added 1 ml. of concentrated hydrochloric acid and 50 ml. of distilled water. The resulting solution is then diluted to 100 ml. with absolute methanol, giving a final concentration of 50 mg. of benzoic acid per liter. Using a slit width of 0.6 mm. and a blank of 50% methanol, which is 0.1N in hydrochloric acid, the absorbance of the benzoic acid is read at 273 mμ. The cells are reversed in position, the absorbances averaged, and the absorptivity is calculated from the equation

$$a = \frac{A}{bc}$$

in which a is the absorptivity of the acid at 273 mμ, A is the average absorbance of the acid solution read at the same wave length, b is the cell length in centimeters, and c is the concentration expressed in grams of acid per liter.

Saponification. A resin sample estimated to contain 0.1 gram of benzoic acid is weighed into a 500-ml. Erlenmeyer flask. It is dissolved in 10 ml. of benzene, and 100 ml. of 0.5N alcoholic potassium hydroxide (made with absolute ethyl alcohol) are added. If it is known that isophthalic acid is absent, the sample may be given the usual 1-hour reflux; otherwise, the flask is warmed in a bath or oven at 45° C. for at least 4 hours. An air condenser is attached, and the sample refluxed 1 hour. Then 150 ml. of dry benzene are added, the flask is stoppered and

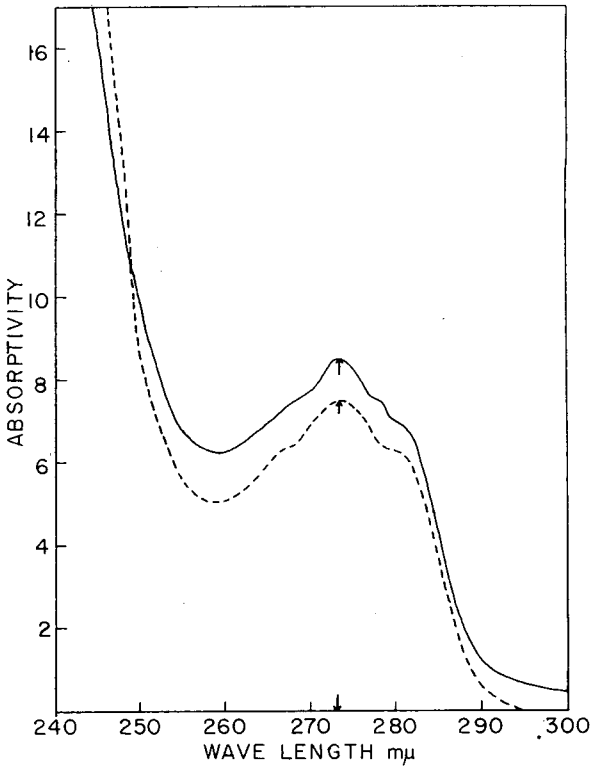

Figure 1. Ultraviolet spectra of benzoic acid in 50% methanol made 0.1N in hydrochloric acid

——— Carried through entire procedure
- - - - Dissolved directly in solvent

Table I. Analysis of Some Alkyd Resins for Benzoic Acid

Type of Alkyd	Benzoic Acid, % Present	Found
Long oil (linseed)	12.5	12.2
	12.5	12.2
	11.2	11.5
	12.4	12.2
	2.9	3.1
Medium oil length (soybean)	16.8	16.4
	15.6	16.2
Short oil length (oxidizing)	15.8	16.2
	14.4	14.4
Special isophthalic acid alkyd (26.7% isophthalic)	9.1	9.9

cooled, and its contents are filtered through a Gooch crucible using benzene for transferring and washing the precipitate. The filtrate is collected in a clean flask; the precipitated salts of the dicarboxylic acids are dried, weighed, and analyzed according to available procedures.

Separation of Fatty Acids from Benzoic Acid. The filtrate mentioned is transferred to a large beaker and the solvents evaporated on a water bath, with water being added periodically to replace the solvents. The resulting alkaline solution is transferred to a 500-ml. separatory funnel and diluted to 300 ml. with water. It is neutralized with concentrated hydrochloric acid and a 5-ml. excess added. The fatty acids are extracted with three 50-ml. portions of carbon tetrachloride, but a fourth extraction is made if the third extract is colored. The solvent layers are combined and the water layer transferred to a 2-liter volumetric flask. The carbon tetrachloride portion is washed in the following manner: 50-ml. portions are washed through three successive separatory funnels each containing 300 ml. of water. The 900 ml. of wash water are combined with the first aqueous layer in the 2-liter flask and diluted to the mark with water. The combined carbon tetrachloride portions may be filtered into a weighed beaker and evaporated for determination of the fatty acids.

Determination of Benzoic Acid. A 500-ml. separatory funnel is filled with sample from the 2-liter flask and allowed to stand. If any further separation occurs, the lower layer is drawn off together with some of the aqueous solution. About 200 ml. of the remaining solution are filtered through a rapid, hardened filter paper, such as Whatman No. 54, and the first 50 ml. are discarded. An exact 100-ml. aliquot of the filtered solution is transferred to a 400-ml. beaker, neutralized with 2*N* potassium hydroxide, and 20 drops in excess are added. A stirring rod is inserted and the sample evaporated to dryness, using an oil bath at 110° C. for the final stages of evaporation, after which the beaker is dried in an oven at 110° C. for 15 minutes. The residue is dissolved in 25 ml. of water, cooled, and neutralized with 6*N* hydrochloric acid with 1 ml. of concentrated hydrochloric acid added in excess. This acidified solution is transferred to a 100-ml. volumetric flask, the beaker rinsed with another 25 ml. of water, followed by methanol, and the solution diluted to the mark with methanol. The flask is thoroughly agitated, and the absorbance of the solution read in the spectrophotometer at 273 mμ, slit width 0.6 mm., using a blank prepared by adding 1 ml. of concentrated hydrochloric acid to 50 ml. of water and diluting to 100 ml. with methanol. The position of the cells is reversed and the readings are repeated. The absorbances are averaged and the benzoic acid is calculated.

$$\% \text{ benzoic acid} = \frac{c_b \times 200 \text{ (based on 100-ml. aliquot)}}{\text{sample weight} \times \text{fraction solids}}$$

$$c_b = \frac{A \text{ (average)}}{a} - (\text{sample weight} \times \text{fraction solids} \times 0.0097)$$

DISCUSSION

In the determination of fatty acids, care must be taken to avoid contaminating the sample with stopcock grease, as all types of these lubricants are removed by carbon tetrachloride and will seriously affect the quantitative measurement. The isolated acids may be tested for contamination with lubricant by their solubility in 95% ethyl alcohol. The lubricants are not soluble and can be filtered off. The use of separatory funnels with Teflon or self-lubricating valves is highly recommended for extractions with carbon tetrachloride.

Slow saponification of the resin sample by warming for several hours prior to refluxing is necessary when isophthalic acid is used in the alkyd resin. If isophthalic acid is precipitated rapidly, fatty acid soaps are entrained and difficulty with the analysis for both the isophthalic and fatty acids is encountered.

Table I gives the results of analyzing alkyd resins. With the exception of the one alkyd containing isophthalic acid, benzoic acid was added in known amounts to all the other samples. The average error in the analysis of all known samples tested is about 2.6%.

LITERATURE CITED

(1) Kappelmeier, C. P. A., *Farben-Ztg.* **40**, 1141 (1935); **41**, 161 (1936); **42**, 561 (1937); *Paint Oil Chem. Rev.* **6**, 10 (1937).

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Determination of Alpha, Beta-Unsaturated Compounds by Reaction with Sodium Sulfite

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As a result of an investigation of chemical methods for the determination of alpha, beta-unsaturated compounds, an acidimetric procedure was developed based on the reaction of these compounds with sodium sulfite. Optimum hydrogen ion concentration for the reaction is attained by the addition of a known amount of sulfuric acid. A stoichiometric quantity of acid is consumed in the reaction and the excess is measured by titration with standard base using an Alizarin Yellow R–Xylene Cyanol FF indicator. Data are presented on the determination of the purity of eight compounds for which this method has been found to give satisfactory results. The advantages and limitations of the sulfite method are discussed and compared with the morpholine method. Also, a guide is given to the selection of the best method for the determination of a compound of this class on the basis of its structure.

AN ACIDIMETRIC method for the determination of alpha, beta-unsaturated compounds, which is based upon the reaction of these compounds with sodium sulfite, has been developed. For convenience, the reaction is illustrated as taking place with the bisulfite ion. In this reaction a substituted sodium sulfonate is formed (*7*) with a corresponding decrease in acidity according to the equation

$$NaHSO_3 + CH_2{=}CH{-}X \longrightarrow NaO_3S{-}CH_2CH_2{-}X \quad (1)$$

where X is any strong electron-attracting group. This reaction has been utilized commercially in the manufacture of anionic surface-active agents (*2*). It has also been used as an analytical procedure by Rosenthaler (*6*) for the determination of maleic acid, but under the conditions which he established he was unable to determine fumaric acid by this method.

In the method of analysis presented here a known excess of sulfuric acid is added to the sodium sulfite reaction mixture. The

decrease in acidity as determined by titration with standard sodium hydroxide to Alizarin Yellow R–Xylene Cyanol FF mixed indicator is a direct measure of the unsaturated compound originally present. Under these conditions sodium sulfite is neutral and a large excess can be used. For maximum reactivity a saturated or nearly saturated solution of the reagent is recommended.

REAGENTS

Isopropyl alcohol, anhydrous commercial grade, Carbide and Carbon Chemicals Co.

Sulfuric acid, approximately 1*N*. Dissolve 49 grams of reagent grade sulfuric acid in 1000 ml. of water.

Table I. Reaction Conditions for Determination of Alpha, Beta-Unsaturated Compounds by Sodium Sulfite Reagent

Compound	Reaction Conditions: Temp., ° C.	Reaction Conditions: Time, min.
Acrylic acid	98[a]	15 to 50
Acrylonitrile	25	5 to 30
Crotonic acid	98[a]	60 to 120
Diethyl fumarate	25[a,b]	15 to 90
Diethyl maleate	25[b,c]	60 to 90
Ethyl acrylate	25[b]	30 to 60
Maleic acid	98[a]	15 to 90
Methyl acrylate	25[b]	15 to 60

[a] Use 15.0 ml. of isopropyl alcohol as a cosolvent.
[b] Place samples and blank in a −10° C. bath for 10 minutes before titration.
[c] Place on mechanical shaker for 15 minutes.

Sodium sulfite, approximately 2*M*. Dissolve 252 grams of anhydrous sodium sulfite in 1000 ml. of distilled water. This reagent should be prepared fresh at least once a week.

Alizarin Yellow R–Xylene Cyanol FF Mixed Indicator. Dissolve 0.1 gram of Alizarin Yellow R and 0.06 gram of Xylene Cyanol FF in 100 ml. of distilled water.

PROCEDURE

To make all sample and blank determinations in duplicate, introduce into each of a sufficient number of glass-stoppered Erlenmeyer flasks 25 ml. of the sodium sulfite reagent by means of a graduated cylinder. For reaction at 98° C. use heat-resistant pressure bottles. Pipet exactly 25.0 ml. of 1*N* sulfuric acid into each flask and add the amount of isopropyl alcohol specified in Table I. Purge the flasks with nitrogen and then stopper. Reserve two of the flasks for blanks. Into each of the other flasks add an amount of sample that contains not more than 15.0 meq. of unsaturated compound. For dilute solutions the samples may be pipetted and the weight calculated from the specific gravity.

Table II. Analysis of Substantially Pure Alpha, Beta-Unsaturated Compounds

Compound	Average Purity, Wt. %: Sodium sulfite method[a]	Average Purity, Wt. %: Other
Acrylic acid	98.4 ± 0.2 (5)	98.7[b]
Acrylonitrile	98.1[c]	...
Crotonic acid	98.7 ± 0.1 (3)	99.1[d]
Diethyl fumarate	99.9 ± 0.1 (8)	99.9[e]
Diethyl maleate	98.6 ± 0.1 (5)	98.6[e]
Ethyl acrylate	99.2 ± 0.1 (6)	99.0[e]
Maleic acid	99.2 ± 0.2 (5)	99.0[d]
Methyl acrylate	98.4 ± 0.1 (7)	98.3[e]

[a] Figures in parentheses represent number of detn.
[b] Modified Kaufmann bromination of potassium salt.
[c] Std. dev. for eight degrees of freedom is 0.09.
[d] Acidity titration.
[e] Saponification.

Allow the samples to react under the conditions specified in Table I. If the reaction is carried out at 98° C. allow the pressure bottles to cool to room temperature before the bottles are uncapped. If specified in Table I, place the samples and blanks in a −10° C. bath for 10 minutes. Add 5 or 6 drops of the Alizarin Yellow R–Xylene Cyanol mixed indicator and titrate the samples and blanks with 0.5*N* sodium hydroxide just to the disappearance of the green color.

Table III. Selection of Reagent for Determination of Alpha, Beta-Unsaturated Compounds of Type R′ H R | | —C=C—X

X	R	R′	Method[a]
—C≡N	H	H	A, B
	Alkyl	H	A
	H	Alkyl	A
	Alkyl	Alkyl	C
—COOH	H	H	A, B
	Alkyl	H	A[b], B[b]
	H	Alkyl	A[b], B
	Alkyl	Alkyl	C
	H	—COOH	B
—COONa	...	...	C[c]
—COOR″	H	H	A, B[d]
	Alkyl	H	A
	H	Alkyl	A
	Alkyl	Alkyl	C
	H	—COOR″	A[e], B[d]
—CONH₂	H	H	A
	H	Alkyl	A[b]
	Alkyl	H	A[b]
	Alkyl	Alkyl	C
—COH	...	...	C[c]
—COR″	...	...	C[c]

[a] A, Morpholine method (*3*); B, sodium sulfite method; C, modified Kaufmann bromination method (*4*).
[b] No experimental data; reaction predicted.
[c] Independent of R and R′.
[d] Only if R″ is methyl or ethyl.
[e] Use conductometric end point determination.

For unsaturated acids it is necessary to determine the acidity of the sample independently by titration with standard sodium hydroxide to phenolphthalein indicator. The procedure for the determination of unsaturation in these acids is identical to that described above, except that the sample titration is usually larger than that of the blank.

DISCUSSION AND RESULTS

The rate of addition of sodium sulfite to alpha, beta-unsaturated compounds is appreciably affected by the pH of the reagent. Figure 1, curve 3, illustrates this effect on the reaction with crotonic acid according to Schenck and Danishefsky (*7*). A similar result is obtained for the reaction with ethyl crotonate at 100° C., curve 2. It is evident from these curves that the optimum range for maximum reactivity is from pH 5.5 to 6.5 In the case of ethyl crotonate at room temperature, curve 1, the apparent conversion over this range of pH is above 100%, indicating the occurrence of secondary reactions.

Two of the secondary reactions which are believed to occur between sodium sulfite and alpha, beta-unsaturated compounds are given by the equations

$$NaHSO_3 \rightleftharpoons NaOH + SO_2 \qquad (2)$$

$$CH_2{=}\overset{H}{\overset{|}{C}}{-}X + SO_2 \underset{\Delta}{\rightleftharpoons} CH_2{-}\underset{SO_2}{\overset{}{CH}}{-}X$$

$$SO_3^{--} + \text{oxidant} \longrightarrow [SO_3]^- + \text{oxidant}^- \qquad (3)$$

$$CH_2{=}CH{-}X + [SO_3]^- \longrightarrow {}^-O_3S{-}CH_2{-}\dot{C}H{-}X$$

$${}^-O_3S{-}CH_2{-}\dot{C}H{-}X + HSO_3^- \longrightarrow {}^-O_3SCH_2CH_2{-}X + [SO_3]^-$$

where *X* is any strong electron-attracting group. The occurrence of the reaction shown in Equation 2 gives high results, because

this method is based upon an acidimetric determination. The net effect of the reaction is an apparent decrease of two equivalents of acidity. This is shown graphically in Figure 1 curve 1. At pH 5.3 and at room temperature the apparent addition of sodium sulfite to ethyl crotonate is 130%. At 100° C., curve 2, this reaction apparently does not occur, presumably because of the thermal instability of the cyclic sulfone reaction product. Also, compounds which are appreciably soluble in the reagent do not undergo this reaction, suggesting that the reaction takes place only at the gas-liquid interface. This reaction is favored at low values of pH, presumably because of the increase of free sulfur dioxide in the gas phase. In some cases this side reaction can be minimized by the addition of a cosolvent such as isopropyl alcohol. Using the reagent as specified, approximately 20.0 ml. of cosolvent can be tolerated before phase separation occurs. Although no literature confirmation of the reaction of sulfur dioxide with alpha, beta-unsaturated compounds has been found, the reaction with butadienes to form cyclic sulfones has been reported (*1*).

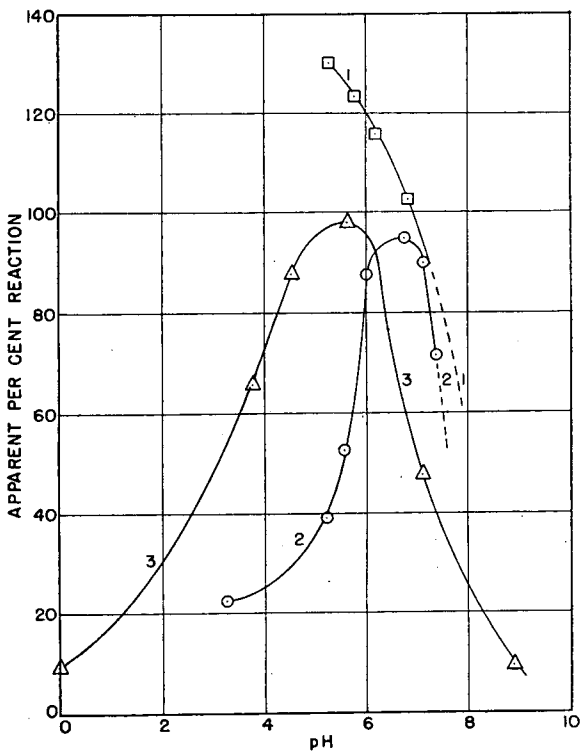

Figure 1. Effect of pH on rate of addition of sodium sulfite to alpha, beta-unsaturated compounds

1. Ethyl crotonate at room temperature
2. Ethyl crotonate at 100° C.
3. Crotonic acid at 100° C. (*7*)

The secondary reaction shown in Equation 3 is similar to the principal nucleophilic reaction in that the same reaction product is formed. However, this reaction proceeds by a free-radical mechanism and is not restricted to conjugated systems (*5*). The occurrence of this reaction therefore prohibits the use of this reagent for the determination of alpha, beta-unsaturation in the presence of olefinic-type unsaturation. As would be expected, this reaction is inhibited by the presence of hydroquinone and the absence of peroxides and molecular oxygen (*5*).

Secondary reactions other than those involving the carbon to carbon double bond are known to occur with this reagent. Among these are the substitution reactions involving a carbon to halogen bond, addition to carbonyls, and saponification of esters. Saponification does not occur at room temperature when the specified reagent, at pH 6.2, is used. However, at 100° C. saponification does take place to an appreciable extent. In the case of ethyl crotonate, Figure 1, curve 2, an apparent 95% reaction is obtained at this temperature. This low result can be attributed to the saponification of 5.0% of this compound. Esters which fail to react quantitatively with the reagent at room temperature cannot be determined by this method.

Among the other reactions that are known to take place with this reagent are the addition to epoxides (*9*), reaction with strong oxidizing and reducing agents, and reaction with vinyl ethers (*8*). As the method is based upon a measurement of the decrease in acidity, a correction must be applied for the presence of mineral and organic acids and inorganic and most organic bases.

A number of compounds which have been successfully determined by the sulfite method are shown in Table II.

CHOICE OF METHOD

From the data obtained in this and other studies of alpha, beta-unsaturation in these laboratories, it is possible to choose the method best suited to the determination of a particular compound from a consideration of its structure. Table III, which is based on reaction-rate studies of each compound, serves as a guide for such selections. In some cases both the morpholine and sodium sulfite methods are applicable and other factors such as the presence of interfering compounds and precision determine the method to be selected. In most cases the sulfite method is more precise than the morpholine method because of the sharpness of the titrimetric end point, but the former is subject to more interferences from side reactions.

LITERATURE CITED

(1) Backer, H. J., Strating, J., and Kool, C. M. H., *Rec. trav. chim.* **58**, 778 (1939).
(2) Caryl, C. R., *Ind. Eng. Chem.* **33**, 731 (1941).
(3) Critchfield, F. E., Funk, G. L., and Johnson, J. B., ANAL. CHEM. **28**, 76 (1956).
(4) Kaufmann, H. P., and Kornmann, O. O., *Z. Untersuch. Lebensm.* **51**, 3 (1926).
(5) Kharasch, M. S., May, E. M., and Mayo, F. R., *J. Org. Chem.* **3**, 175 (1938).
(6) Rosenthaler, L., *Pharm. Acta Helv.* **17**, 196 (1942).
(7) Schenck, R. T. E., and Danishefsky, I., *J. Org. Chem.* **16**, 1683 (1951).
(8) Siggia, S., IND. ENG. CHEM., ANAL. ED. **19**, 1025 (1947).
(9) Swan, J. D., ANAL. CHEM. **26**, 878 (1954).

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CORRECTIONS

Concept of Polarographic Currents Limited by Rate of a Chemical Reaction and Some of Its Applications

In the article on "Concept of Polarographic Currents Limited by Rate of a Chemical Reaction and Some of Its Applications" [ANAL. CHEM. **27**, 1712 (1955)] in the second column, the fifth line under Equation 1 should read: $= \frac{3}{5} 0.85 (mt)^{2/3}$.

KAREL WIESNER

Slow Precipitation Processes

In the article on "Slow Precipitation Processes. Application of Precipitation from Homogeneous Solution to Liquid-Solid Distribution Studies" [ANAL. CHEM. **27**, 1704 (1955)], Equation 6 should have been written:

$$\ln \frac{Ra_{initial}}{Ra_{final}} = \lambda \ln \frac{Ba_{initial}}{Ba_{final}}$$

LOUIS GORDON

Determination of Alpha, Beta-Unsaturated Compounds by Reaction with Morpholine

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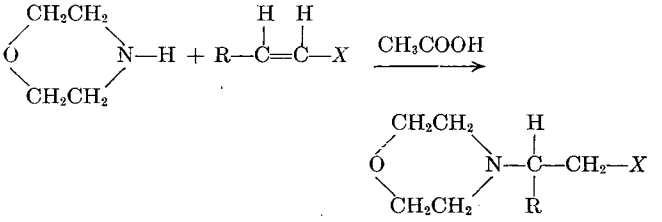
With the increasing commercial interest in alpha, beta-unsaturated compounds, such as acrylic acid and its derivatives, satisfactory chemical methods were sought for the determination of these compounds. The usual halogenation methods based on the reaction of the unsaturated group are not generally applicable. It was found that this class of compounds react quantitatively with morpholine in the presence of acetic acid catalyst to form tertiary amines. After the excess morpholine reacts with acetic anhydride to convert to the amide, which is neutral in the reaction medium, the tertiary amine is titrated with methanolic hydrochloric acid, using an indicator. In a few cases in which the tertiary amine is too weak to titrate in this manner, a conductometric titration is used. The method has been successfully applied to 15 compounds with good accuracy and precision. Interfering compounds and those which fail to react are discussed.

ALPHA, beta-unsaturated compounds are of considerable commercial importance, principally because of their ease of polymerization. Although several of these compounds are being used extensively for the manufacture of commercial polymers, few satisfactory methods are available for their determination. In general compounds with a carbon to carbon double bond adjacent to a strong electron-attracting group are difficult, if not impossible, to halogenate quantitatively. The decreased reactivity of the double bond in these compounds with halogens can be attributed to the increase in positive character of the unsaturation due to the proximity of a strong electron-attracting group. Among the halogenation procedures that have been applied to some compounds of this class are the mercury catalyzed bromate-bromide reagent of Lucas and Pressman (*6*), the pyridine sulfate dibromide reagent of Rosenmund, Kuhnheim, Rosenberg-Gruszynski, and Rosetti (*8*), and the modification of Rowe, Furnas, and Bliss (*9*). A modification of the Kaufmann bromine-bromide reagent (*5*) has been used in these laboratories for the determination of a few compounds of this class. In general this reagent cannot be used for the determination of the unsaturation of compounds in which the carbon to carbon double bond is adjacent to groups such as nitrile, carboxyl, amide, sulfonic acid, nitro, and carbalkoxy.

Methods other than those based upon halogenation reactions have been applied to certain alpha, beta-unsaturated compounds. Catalytic hydrogenation procedures (*7, 10, 11*) have been used, but generally the poor precision and accuracy of these methods limit their usefulness. Boer and Koogman (*2*) used a standardized stream of ozone for the titration of unsaturation and present data for a few compounds of this class. Beesing, Tyler, Kurtz, and Harrison (*1*) determined a number of alpha, beta-conjugated compounds by an alkaline-catalyzed reaction with dodecanethiol. This method, which is based upon a nucleophilic addition to the carbon to carbon double bond, appears to be the only method published which is specifically designed for the determination of this class of compounds. A method based upon reaction of these compounds with sodium sulfite has been developed, and the selection of the method most applicable to the determination of conjugated unsaturated compounds as a function of structure also has been given (*3*).

Although the reaction of primary and secondary amines with alpha, beta-unsaturated compounds has been known for several years (*4, 12*), no analytical application of this principle has been published. In the method presented here an excess of morpholine, a secondary amine, reacts in the presence of acetic acid catalyst with the unsaturated compound according to the following equation:



where X is any strong electron-attracting group. After the reaction is complete the excess morpholine is acetylated with acetic anhydride in acetonitrile medium to form the corresponding amide and acetic acid. The tertiary amine formed in the reaction is titrated with standard alcoholic hydrochloric acid to methyl orange–Xylene Cyanol mixed indicator. Under these conditions the amide and acetic acid are neutral and the amount of tertiary amine formed is a direct measure of the alpha, beta-unsaturated compound originally present.

APPARATUS

All potentiometric titrations were performed using a Leeds & Northrup pH meter equipped with glass and calomel electrodes. Conductometric titrations were made using a Model RCM 15 Serfass direct-reading conductance bridge. With this instrument a dip-type conductivity cell with platinized-platinum electrodes having a cell constant of approximately 0.1 cm.$^{-1}$ was used.

REAGENTS

Acetonitrile, commercial grade, Carbide and Carbon Chemicals Co.

Acetic Acid Solution. Mix one part of glacial acetic acid, Grasselli reagent grade, with one part of distilled water.

Acetic Anhydride, commercial grade, Carbide and Carbon Chemicals Co.

Methanol, anhydrous, commercial grade, Carbide and Carbon Chemicals Co.

Morpholine, anhydrous, commercial grade, Carbide and Carbon Chemicals Co.

Standard 0.5*N* Hydrochloric Acid in Methanol. Transfer 85 ml. of 6*N* hydrochloric acid to a 1000-ml. volumetric flask and dilute to volume with methanol. Standardize by titrating exactly 40.0 ml. of the solution with standard 0.5*N* sodium hydroxide using phenolphthalein indicator. This titrant can best be dispensed from an automatic buret assembly and should be standardized daily. For the change of normality with increase in temperature use $\Delta N/°$ C. $= -0.0005$.

Methyl Orange–Xylene Cyanol Mixed Indicator. Dissolve 0.15 gram of methyl orange and 0.08 gram of Xylene Cyanol FF in 100 ml. of distilled water.

PROCEDURE

Indicator Method. By means of a graduated cylinder or dispensing buret add 10 ml. of morpholine to each of two 250-ml. glass-stoppered Erlenmeyer flasks. For reactions at 98° ± 2° C. use heat-resistant pressure bottles. Reserve one of the flasks as a blank. Into the other flask introduce an amount of sample containing not more than 23 meq. of the unsaturated compound.

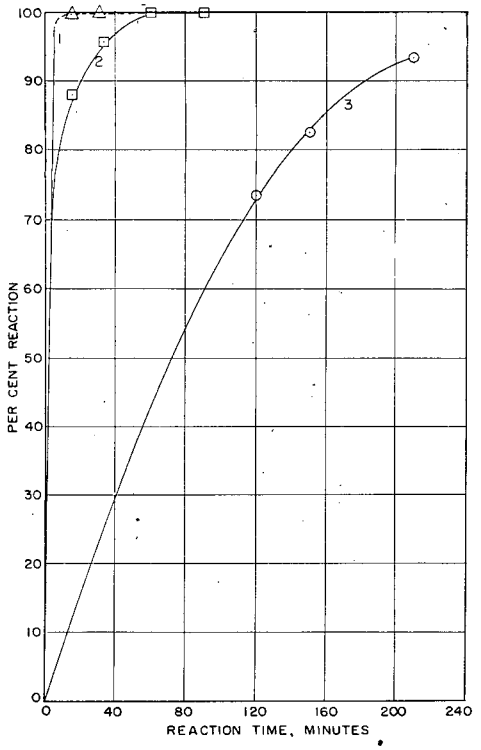


Figure 1. Effect of acid catalysts on reaction of morpholine with ethyl crotonate

1. Acetic acid catalyst
2. Hydrochloric acid catalyst
3. No catalyst

For substantially pure material weigh to the nearest 0.1 mg. For dilute solutions the sample may be added by means of a pipet and the sample weight calculated from the specific gravity. To each flask add 7.0 ml. of acetic acid solution, unless otherwise specified, and the amount of cosolvent specified in Table I. Allow both the sample and the blank to stand for the time and at the temperature specified in Table I. If elevated temperatures are used, carefully cool the pressure bottles to room temperature. Add 50 ml. of acetonitrile to each flask by means of a graduated cylinder. While constantly swirling the flask add 20 ml. of acetic anhydride to both the sample and blank from a suitable graduated cylinder or dispensing buret and stopper. Allow to cool to room temperature. Add 5 or 6 drops of methyl orange–Xylene Cyanol mixed indicator and titrate with standard 0.5*N* methanolic hydrochloric acid to the disappearance of the green color. A Fisher titrating light or similar device greatly facilitates the selection of the end point.

Conductometric Method. Follow the above procedure, except limit the sample to less than 10.0 meq. of unsaturated compound. Titrate the blank using the indicator method. Quantitatively transfer the contents of the sample flask to a 250-ml. tall-form beaker. Immerse the conductivity cell in the solution and add methanol to cover the electrodes. Titrate with standard 0.5*N* hydrochloric acid in methanol, taking conductance measurements at three or four points on each side of the anticipated end point. The end point is selected from a graphical plot of these data.

DISCUSSION

Acid Catalysis. The esters of acrylic acid react so readily with morpholine that these compounds can be determined without the addition of catalytic substances. As shown in Figure 1, curve 3, the reaction with ethyl crotonate proceeds only with difficulty at elevated temperatures. In the presence of a catalytic amount of hydrochloric acid, curve 2, this reaction is quantitative in 60 minutes at 98° C. Acetic acid is even more effective, curve 1, and does not interfere in the subsequent determination of the tertiary amine reaction product.

Figure 2 shows the effect of acetic acid on the reaction rate of morpholine with methyl methacrylate. From this curve it can be seen that by the addition of 3 ml. of catalyst solution containing 50% acetic acid to the reaction mixture a threefold increase is effected in the rate of reaction. At concentrations of acid greater than 55%, morpholinium acetate is precipitated with subsequent depletion of the reagent. In the procedure finally adopted 7.0 ml. of 50% acetic acid has been specified, because in most cases the maximum rate of reaction is obtained with this volume of 50% acetic acid.

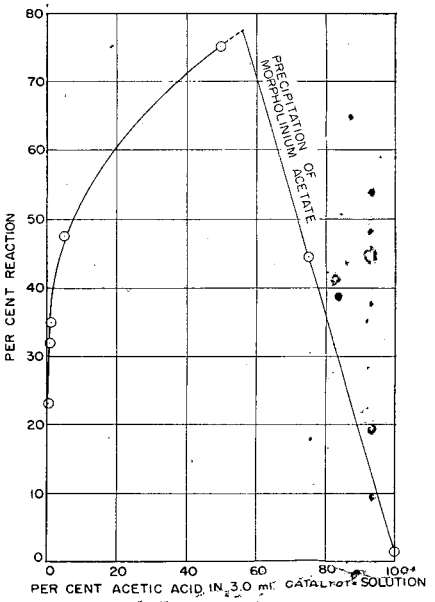


Figure 2. Effect of acetic acid concentration on reaction of morpholine with methyl methacrylate

Reaction time, 15 minutes at room temperature

Effect of Structure upon Reactivity. From the data obtained in this investigation several important generalizations relating structure and reactivity can be made. In general the compounds that react most readily with the reagent are acrylic-type compounds possessing the structure H_2—C═C(H)—*X*, where *X* is any strong electron-attracting group (meta-orienting group). Of the compounds investigated the following sequence of reac-

Table I. Reaction Conditions for Alpha, Beta-Unsaturated Compounds by Morpholine Reaction

Compound	Reaction Conditions: Time, min.	Reaction Conditions: Temp., ° C.
Acrylamide	5 to 120	25
Acrylic acid	15 to 120[a]	98
Acrylonitrile	5 to 60	25
Allyl cyanide (3-butenenitrile)	30 to 60	98
Butyl acrylate	5 to 60	25
Diethyl fumarate	30 to 90[b]	25
Di(2-ethylhexyl) maleate	45 to 90[b],	25
Diethyl maleate	30 to 90[c]	25
Ethyl acrylate	5 to 60	25
2-Ethylbutyl acrylate	5 to 30	25
Ethyl crotonate	15 to 60	98
2-Ethylhexyl acrylate	5 to 60	25
Methacrylonitrile	120 to 240	98
Methyl acrylate	5 to 60	25
Methyl methacrylate	40 to 80	98

[a] Use 10 ml. of methanol cosolvent.
[b] Use conductometric titration procedure.
[c] Use 40 ml. of methanol cosolvent and 2 ml. of acetic acid solution.

Table II. Analysis of Substantially Pure Unsaturated Compounds

Compound	Average Purity[a], Wt. % Morpholine method	Other
Acrylamide	100.0 ± 0.1 (5)	...
Acrylic acid	98.6 ± 0.1 (4)	98.7[b]
Acrylonitrile	98.3[c]	...
Allyl cyanide	98.3 ± 0.1 (4)	98.3[d]
Butyl acrylate	99.8 ± 0.1 (4)	99.8[e]
Diethyl fumarate	99.5 ± 0.2[b] (2)	99.9[e]
Di(2-ethylhexyl) maleate	99.7 ± 0.2[f] (2)	100.0[e]
Diethyl maleate	98.4 ± 0.2[f] (2)	98.6[e]
Ethyl acrylate	99.2 ± 0.2 (4)	99.0[e]
2-Ethylbutyl acrylate	99.6 ± 0.1 (4)	99.5[e]
Ethyl crotonate	100.0 ± 0.1 (3)	99.9[e]
2-Ethylhexyl acrylate	99.0 ± 0.1 (4)	99.0[e]
Methacrylonitrile	97.9 ± 0.2 (3)	...
Methyl acrylate	98.7 ± 0.2 (4)	98.3[e]
Methyl methacrylate	97.8 ± 0.1 (3)	98.8[e]

[a] Figures in parentheses represent number of determinations.
[b] Bromination of potassium salt.
[c] Std. dev. for five degrees of freedom is 0.11.
[d] Bromination by a modified Kaufmann procedure.
[e] Saponification.
[f] Conductometric end point.

tivity with morpholine, as a function of the electron-attracting group of acrylic compounds, was found.

$$-C{\equiv}N \gtrsim -\overset{O}{\overset{\|}{C}}-OR \gg -\overset{O}{\overset{\|}{C}}-OH > -\overset{O}{\overset{\|}{C}}-R' \geq -\overset{O}{\overset{\|}{C}}-ONa$$

where R is alkyl and R′ is alkyl or hydrogen.

Attempts to determine the sodium or potassium salts of alpha, beta-unsaturated acids and alpha, beta-unsaturated aldehydes and ketones by this method were unsuccessful. These compounds, which are relatively unreactive with morpholine, react smoothly and completely with brominating reagents, such as the modified Kaufmann reagent.

The substitution of alkyl groups on either the alpha or beta-carbon atoms of acrylic compounds has a marked retarding effect upon the rate of reaction with morpholine. For the determination of these compounds elevated temperatures must be employed. The effect of these alkyl groups can be attributed to their electron-releasing tendencies. The net effect of alpha or beta-alkyl substitution is an increase of electron density about the beta carbon atom, thereby decreasing the possibility of attack by a nucleophilic reagent. For the same reason there is an increase in the susceptibility of attack by electrophilic reagents such as bromine. The retarding effect of alkyl substituents on the reactivity of alpha, beta-unsaturated compounds with morpholine is as follows.

$$\alpha,\beta \gg \alpha > \beta$$

Of the compounds investigated that possessed both alpha and beta substituents, no detectable reaction was observed even at elevated temperatures. As was pointed out, these compounds react more readily with brominating reagents and many can be determined in this manner.

Certain beta, gamma-unsaturated compounds, such as allyl cyanide, Table II, are isomerized to the corresponding alpha, beta-unsaturated compound under the conditions of the reaction and can be determined by this method. Allyl cyanide is the only compound investigated that can be determined by both the usual brominating reagents and by reaction with morpholine.

Table II lists 15 compounds which have been analyzed successfully by the morpholine method.

Ratio of Morpholine to Unsaturated Compounds. Figure 3 shows the effect of excess reagent on the reactivity of morpholine with acrylonitrile at a fixed acetic acid to morpholine mole ratio of 0.5 to 1 and at a fixed reaction time of 5 minutes at room temperature. From this curve it is apparent that a reagent to reactant mole ratio of at least 2.5 to 1 is necessary to obtain quantitative reaction in the shortest length of time. To provide a reasonable amount of reaction medium and a margin of safety, a mole ratio of 5 to 1 is used.

Effect of Tertiary Amine Strength. From the potentiometric titration curves shown in Figure 4 it can be seen that the sharpness of the equivalence point is appreciably affected by the nature of the tertiary amine formed in this reaction. The effect is particularly accentuated in the case of the reaction product of morpholine with maleic and fumaric esters. As shown in curve 4, this amine is too weak to be determined by either indicator or potentiometric methods. The decreased basicity of these amines can be attributed to the fact that the tertiary nitrogen is alpha to a strong electron-attracting group. Although an acidic sol-

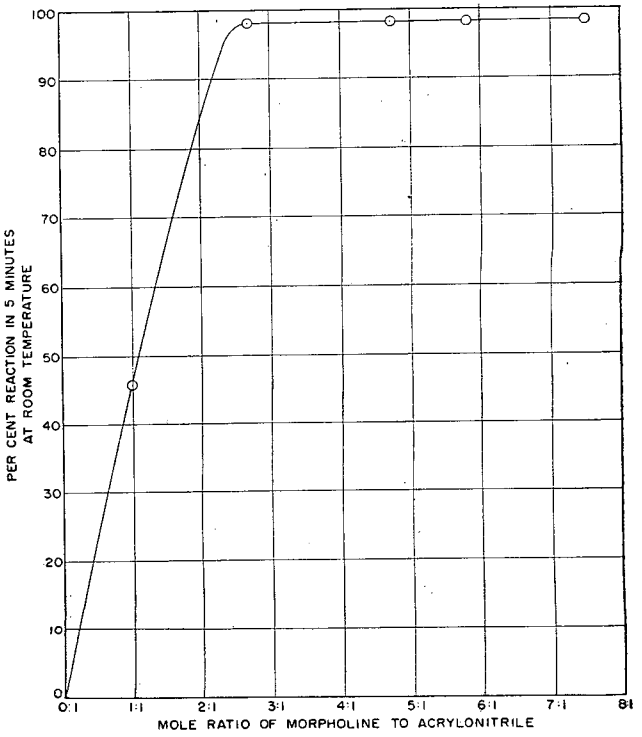


Figure 3. Effect of mole ratio on reaction of morpholine with acrylonitrile

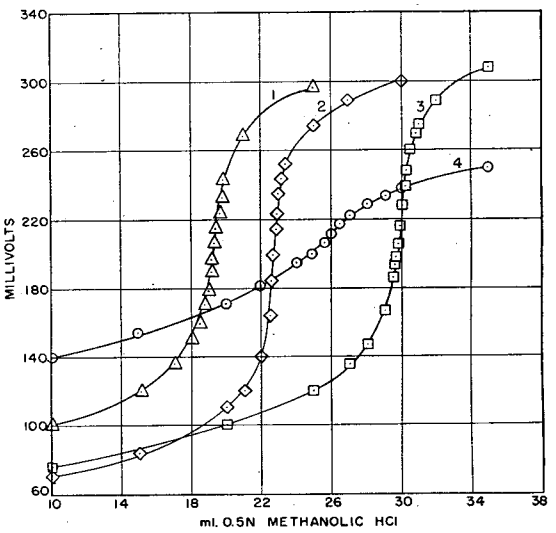


Figure 4. Potentiometric titration curves of tertiary amines formed from reaction of morpholine alpha, beta-unsaturated compounds

1. Acrylonitrile
2. Methyl methacrylate
3. Acrylic acid
4. Diethyl maleate

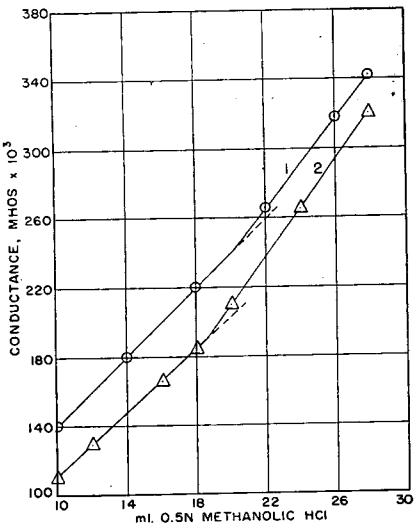


Figure 5. Conductometric titration curves of tertiary amines formed from reaction of morpholine with diethyl fumarate and di(2-ethylhexyl) maleate

1. Diethyl fumarate 2. Di(2-ethylhexyl) maleate

vent, such as glacial acetic acid, enhances the basicity of these amines, it also enhances the basicity of amides to the extent that they interfere in the titration. These weak tertiary amines can be determined, however, by employing a conductometric titration procedure. Figure 5 shows the titration of the amines formed by the reaction of morpholine with diethyl fumarate and di(2-ethylhexyl) maleate. This method of end-point determination can be employed in this case, because straight lines are obtained on each side of the equivalence point. In the selection of the end point only these lines are considered and the points in the vicinity of the end point are ignored. With this procedure it is necessary to use a smaller sample size, so that the net titration does not exceed 20 ml. of titrant. Because a separate curve must be plotted for each determination this method is not readily adaptable to routine determinations. However, in the hands of an experienced operator results within ± 0.2% have been obtained in the determination of the purity of maleic and fumaric esters.

INTERFERENCES

As this method is based upon a nonaqueous titration of the tertiary amine formed in the reaction, it is subject to interference from acid and basic constituents present in the sample. Acids with ionization constants in water greater than 2×10^{-2}, tertiary amines, and inorganic bases titrate quantitatively under these conditions and a correction may be applied.

Most epoxides react quantitatively with the reagent to form tertiary amines, which also are basic under the conditions of the titration. A method for the determination of epoxides, based on this reaction, has been developed in these laboratories.

Large quantities of aldehydes, ketones, and anhydrides may interfere by depleting the reagent. Organic halides react with morpholine to liberate halogen acids and therefore cannot be tolerated.

Most compounds with unsaturation not conjugated to a strong electron-attracting group (isolated unsaturation) and most alpha, beta-unsaturated compounds substituted in both the alpha and beta positions do not react.

LITERATURE CITED

(1) Beesing, D. W., Tyler, W. P., Kurtz, D. M., and Harrison, S. A. ANAL. CHEM. 21, 1073 (1949).
(2) Boer, H., and Koogman, E. C., *Anal. Chim. Acta* 5, 550 (1951).
(3) Critchfield, F. E., and Johnson, J. B., ANAL. CHEM. 27, 73 (1955).
(4) Ford, J. H., Buc, S. R., and Greiner, J. W., *J. Am. Chem. Soc.* 69, 844 (1947).
(5) Kaufmann, H. P., and Kornmann, *Z. Untersuch. Lebensm.* 51, 3, (1926).
(6) Lucas, H. J., and Pressman, D., IND. ENG. CHEM., ANAL. ED. 10, 140 (1938).
(7) Ogg, C. L., and Cooper, F. J., ANAL. CHEM. 21, 1400 (1949).
(8) Rosenmund, K. W., Kuhnheim, W., Rosenberg-Gruszynski, D., and Rosetti, H., *Z. Untersuch. Nahr.-u. Genussm.* 46, 154 (1923).
(9) Rowe, R. G., Furnas, C. C., Bliss, H., IND. ENG. CHEM., ANAL. ED. 16, 371 (1944).
(10) Savacool, R. V., and Ullyot, G. E., ANAL. CHEM. 24, 714 (1952).
(11) Vandenheuvel, F. A., *Ibid.*, 24, 847 (1952).
(12) Whitmore, F. C., Mosher, H. S., Adams, R. R., Taylor, R. B., Chapin, E. C., Weisel, C., and Yanko, W., *J. Am. Chem. Soc.* 66, 725 (1944).

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4-Methyl- and 4-Isopropyl-1,2-Cyclohexanedionedioxime

Gravimetric Reagents for Nickel and Palladium

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Since it is somewhat inconvenient at the present time to synthesize seven-membered alicyclic *vic*-dioximes, it seemed desirable to study the more easily prepared substituted-1,2-cyclohexanedionedioximes as analytical reagents. 4-Methyl-1,2-cyclohexanedionedioxime was found to be an excellent reagent for the gravimetric determination of both nickel and palladium. It is water-soluble, quantitatively precipitates nickel at pH 3, forms a precipitate free from contamination of excess reagent which filters easily and does not creep, and has a high equivalent weight. 4-Isopropyl-1,2-cyclohexanedionedioxime, while being somewhat less soluble in water, is especially useful for the determination of small amounts of nickel and palladium.

THE desirability and applicability of water-soluble *vic*-dioximes as analytical reagents have been discussed by Voter and Banks (4). They pointed out that compounds with oxime groups attached to the adjacent carbon atoms of six- or seven-membered alicyclic rings possess several advantages over other *vic*-dioximes. These alicyclic *vic*-dioximes have a much higher molar solubility in water than do straight-chain aliphatic *vic*-dioximes of similar or even lower molecular weights. Other advantages are that they are sensitive for the detection of nickel and palladium in submicrogram amounts, that they quantitatively precipitate these metal ions from acid solutions, and that they have high equivalent weights.

1,2-Cyclohexanedionedioxime (nioxime) is an excellent rea-

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gent for the gravimetric determination of palladium (*6*), but an empirical factor is required in the determination of nickel (*5*). 1,2-Cycloheptanedionedioxime (heptoxime) would very closely approach being an ideal analytical reagent (*1*, *4*) if it were not for the fact that the synthesis of seven-membered alicyclic rings is somewhat inconvenient at the present time. Alicyclic *vic*-dioximes with less than six carbon atoms in the ring are very pH dependent in their analytical applications, whereas those with greater than seven carbon atoms in the ring are relatively insoluble in water (*3*).

It would appear then that a study of substituted alicyclic *vic*-dioximes which contain a six-membered ring would have the greatest possibility of yielding better analytical reagents. With this goal in mind the following alkyl-1,2-cyclohexanedionedioximes (alkylnioximes) were prepared (*2*) and studied: 4-methyl-, 4-isopropyl-, 4-*tert*-amyl-, 4-(1,1,3,3-tetramethylbutyl)-, 3-methyl-, 3-ethyl-, and 3,6-dimethyl-1,2-cyclohexanedionedioxime. Of the compounds studied only the 4-methylnioxime appeared to have been described (*7*), and this compound, as well as the 4-isopropylnioxime, appeared to be promising as gravimetric reagents for both nickel and palladium.

4-Methylnioxime was found to be an excellent reagent for the gravimetric determination of both nickel and palladium. In the determination of nickel this reagent has more favorable attributes than any *vic*-dioxime suggested to date. It is water-soluble (3.4 grams per liter at 25° C.), quantitatively precipitates nickel as a scarlet-colored complex down to pH 3, forms a precipitate free from contamination of excess reagent which filters easily and does not tend to creep, and has a high equivalent weight. The only *vic*-dioxime known to possess equivalent virtues is heptoxime, but the cost of preparing this reagent is much greater than for 4-methylnioxime. 4-Methylnioxime is equally useful for the gravimetric determination of palladium.

4-Isopropylnioxime while being less soluble (0.75 gram per liter at 25° C.) is still sufficiently soluble to be a very useful reagent for the gravimetric determination of small amounts of nickel and palladium.

REAGENTS

4-Methylnioxime. A 0.34% aqueous solution was used. This saturated solution is stable indefinitely.

4-Isopropylnioxime. A 0.075% aqueous solution was used This saturated solution is stable indefinitely.

Standard Nickel Solution. This was prepared as previously described (*5*) and standardized with heptoxime (*4*).

Standard Palladium Solution. This was prepared as previously described and standardized with nioxime (*6*).

Other Solutions. A 20% solution of ammonium acetate, a 33% solution of citric acid, a 10% solution of sodium sulfite, and a 50% solution of ammonium thiocyanate were prepared from reagent grade salts and filtered.

All of the salts used in the interference studies were of reagent grade quality.

SENSITIVITY

Tests made in the same way as reported for nioxime with nickel (*5*) and with palladium (*6*) showed that both 4-methylnioxime and 4-isopropylnioxime have sensitivities for these ions which are identical with those for nioxime.

DETERMINATION OF NICKEL

4-Methylnioxime. Nickel is quantitatively precipitated as bis(4-methyl-1,2-cyclohexanedionedioximato-*N*,*N'*)nickel(II) in the pH range of 3 to 7. While quantitative precipitation of nickel does occur at pH values above 7, the precipitate formed under these conditions is more voluminous and rather difficult to handle. The presence of some buffer such as ammonium acetate is necessary, because hydrogen ions are released as precipitation occurs. Both synthetic and National Bureau of Standards samples containing from 11 to 60 mg. of nickel were analyzed by the recommended procedure and the results are shown in Table I. Up to 300% excess of precipitant may be present without causing an error. The factor for nickel in bis(4-methyl-1,2-cyclohexanedionedioximato-*N*,*N'*)nickel(II) [$(C_7H_{11}O_2N_2)_2Ni$] is 0.1590.

Table I. Gravimetric Determination of Nickel with 4-Methylnioxime

Sample	No. of Detns.	Nickel Actual, mg.	Nickel Found, mg.	Error, mg.
$Ni(ClO_4)_2$	4	1.20	1.23 ± 0.05	+0.03
$Ni(ClO_4)_2$	4	5.99	5.98 ± 0.08	−0.01
$Ni(ClO_4)_2$	4	24.0	24.0 ± 0.08	±0.0
$Ni(ClO_4)_2$	4	59.9	59.6 ± 0.1	−0.3
		%	%	%
N.B.S. cast iron No. 115[a]	3	15.89	15.85 ± 0.05	−0.04
N.B.S. 18 chromium-9 nickel steel No. 101c[a]	2	9.27	9.28 ± 0.01	+0.01
N.B.S. nickel steel No. 33c[a]	3	3.28	3.28 ± 0.01	±0.00
N.B.S. nickel-molybdenum steel No. 111a[a]	3	1.75	1.75 ± 0.01	±0.00
N.B.S. chromium-nickel-molybdenum steel No. 139[a]	3	0.563	0.567 ± 0.007	+0.004
N.B.S. aluminum-base alloy No. 85a[a]	2	0.41	0.41 ± 0.00	±0.00

[a] Ten grams of citric acid added.

Table II. Effect of Various Cations on Gravimetric Determination of Nickel with 4-Methyl- and 4-Isopropylnioxime

Cation Added[a]	4-Methylnioxime: Nickel found[b], mg.	4-Methylnioxime: Error, mg.	4-Isopropylnioxime: Nickel found[c], mg.	4-Isopropylnioxime: Error, mg.
Aluminum[d]	37.9	+0.1	2.04	−0.06
Ammonium	37.7	−0.1	2.11	+0.01
Antimony(III)[d]	37.7	−0.1	2.05	−0.05
Barium	37.6	−0.2	2.13	+0.03
Beryllium	37.7	−0.1	2.06	−0.04
Bismuth[d]	37.6	−0.2	2.06	−0.04
Cadmium	37.8	±0.0	2.09	−0.01
Calcium	37.8	±0.0	..	..
Cerium(III)	37.9	+0.1	2.04	−0.06
Chromium(III)[d]	37.8	±0.0	2.07	−0.03
Cobalt(II)[e]	37.9	+0.1	2.29	+0.19
Cobalt(III)[f]	37.9	+0.1	2.11	+0.01
Copper(II)[e]	37.9	+0.1	2.26	+0.16
Copper(I)[g]	38.0	+0.2	2.06	−0.04
Iron(II)[h]	37.9	+0.1	..	..
Iron(III)[d]	37.8	±0.0	2.04	−0.06
Lanthanum	37.9	+0.1	2.09	−0.01
Lead(II)	37.7	−0.1	2.13	+0.03
Lithium[i]	37.8	±0.0	..	..
Magnesium	37.6	−0.2	..	..
Manganese(II)	37.8	±0.0	2.08	−0.02
Palladium(II)[j]	37.7	−0.1	..	..
Platinum(II)	37.7	−0.1	2.15	+0.05
Rhodium(III)	..	..	2.14	+0.04
Ruthenium(III)	37.9	+0.1	2.16	+0.06
Silver	37.8	±0.0	2.06	−0.04
Strontium	37.9	+0.1	..	..
Thorium	37.9	+0.1	2.06	−0.04
Titanium(III)[d]	37.7	−0.1	..	..
Uranium(VI)	37.8	±0.0	2.04	−0.06
Vanadium(IV)	37.7	−0.1	2.05	−0.05
Zinc	37.6	−0.2	..	..

[a] 200 mg. of cation present for each determination with 4-methylnioxime except where otherwise noted; 1 gram of cation present for each determination with 4-isopropylnioxime.
[b] Nickel present 37.8 mg.
[c] Nickel present 2.10 mg.
[d] Complexed with tartrate.
[e] 20 mg. of the cation present for determination with 4-methylnioxime.
[f] Complexed with cyanide.
[g] Complexed with thiocyanate after reduction with sulfite.
[h] Hydroxylammonium chloride added.
[i] Potassium and sodium gave identical results.
[j] Ammonium hydroxide added to pH 8 for precipitation.

4-Isopropylnioxime. The optimum conditions for quantitatively precipitating nickel as bis(4-isopropyl-1,2-cyclohexanedionedioximato-*N*,*N'*)nickel(II) were found to be the same as those for 4-methylnioxime. The amount of nickel conveniently determined is less for 4-isopropylnioxime than for 4-methylnioxime; however, the higher equivalent weight and the lower solubility of the nickel complex of the former reagent make it particularly suitable for the determination of small quantities of nickel. Excess reagent does not affect the results. The factor for nickel in bis(4-isopropyl-1,2-cyclohexanedionedioximato-*N*,*N'*)nickel(II) [$(C_9H_{16}O_2N_2)_2Ni$] is 0.1380.

Interferences. No interference in the determination of nickel with 4-methylnioxime was observed from the presence of 1 gram

Table III. Gravimetric Determination of Palladium with 4-Methyl- and 4-Isopropylnioxime

Reagent	No. of Detns.	Palladium, Mg. Taken	Palladium, Mg. Found	Error, Mg.
4-Methylnioxime	3	6.25	6.2 ± 0.1	±0.0
	3	12.5	12.6 ± 0.1	+0.1
	3	25.0	25.1 ± 0.1	+0.1
	3	50.0	49.8 ± 0.2	−0.2
4-Isopropylnioxime	3	1.25	1.18 ± 0.05	−0.07
	3	2.50	2.46 ± 0.05	−0.04
	3	3.75	3.76 ± 0.03	+0.01
	3	5.00	5.03 ± 0.04	+0.03

of the following anions: acetate, chloride, nitrate, perchlorate, sulfate, tartrate, citrate, sulfosalicylate, and thiocyanate. The first six of the above anions were also checked and found not to interfere in the determination of nickel with 4-isopropylnioxime.

The results of a study of the effect of various cations are shown in Table II. Aluminum, antimony(III), bismuth, chromium(III), and iron(III) may be effectively masked with tartrate. Cobalt, copper, and palladium must be masked with cyanide, thiocyanate, and ammonia, respectively.

Recommended Procedure. Adjust the volume of the solution, containing from 5 to 50 mg. of nickel, to about 250 ml. Add sufficient complexing agent to mask any interfering ions present. Adjust the pH to near 1 by adding hydrochloric acid, and heat the solution to 60° C. Add 20 ml. of the 4-methylnioxime solution (or 100 ml. of the 4-isopropylnioxime solution) for each 10 mg. of nickel present. Add ammonium hydroxide dropwise to the first appearance of a pink color, and then add 25 ml. of the ammonium acetate solution dropwise. Digest the precipitate for one half hour at 60° C., and filter through a weighed filter crucible of medium porosity. Wash the precipitate with hot water, dry at 105° C. for 2 hours, and weigh.

DETERMINATION OF PALLADIUM

4-Methylnioxime. Palladium is quantitatively precipitated as bis(4-methyl-1,2-cyclohexanedionedioximato-*N*,*N'*)palladium(II) in the pH range 0.7 to 5. Various methods for the precipitation of the palladium complex were investigated, but the best procedure appeared to be similar to that suggested by Voter, Banks, and Diehl (*6*) for use with nioxime. Several synthetic samples were analyzed by the recommended procedure, and the results are shown in Table III.

4-Isopropylnioxime. From 1 to 5 mg. of palladium can be accurately determined with 4-isopropylnioxime by the recommended procedure, as is shown by the results in Table III.

Interferences. One gram of acetate, chloride, nitrate, perchlorate, sulfate, sulfosalicylate, or tartrate gave no significant interference in the determination of palladium with either 4-methylnioxime or 4-isopropylnioxime. The presence of 1 gram of the following cations gave no greater effect than that listed in Table II for the corresponding determination of nickel: aluminum, antimony(III), barium, beryllium, bismuth (chloride absent), cadmium, calcium, cerium(III), chromium(III), cobalt(II), copper(II), iron(II), iron(III) (tartrate present), lanthanum, lead(II), lithium, magnesium, manganese(II), platinum(II), potassium, rhodium(III), ruthenium(III), silver (chloride absent), sodium, strontium, thorium, titanium(III), uranium(VI), vanadium(IV), and zinc. In addition, iridium(III), nickel, niobium (oxalate present), and zirconium were found to have negligible effect. Both 4-methyl- and 4-isopropylnioxime reduce gold to the metal. Large amounts of highly charged ions such as aluminum and thorium tend to give low results for small amounts (2 mg.) of palladium, probably through ionic strength effects. Dilution of the solution before precipitation effectively reduces this error.

Recommended Procedure. Adjust the volume of the solution, containing from 5 to 50 mg. of palladium, to about 250 ml. Add a complexing agent if necessary. Adjust the pH to from 1 to 1.5 and heat the solution to near 60° C. Add slowly from a pipet with stirring 10 ml. of the 4-methylnioxime solution (or 50 ml. of the 4 isopropylnioxime solution) for each 10 mg. of palladium present. Digest the precipitate for one half hour at 60° C. and filter through a weighed filter crucible of medium porosity. Wash the precipitate with hot water, dry at 105° C. for 2 hours, and weigh. The factor for palladium is 0.2558 if 4-methylnioxime is used and 0.2255 if 4-isopropylnioxime is used. As little as 1 mg. of palladium can be determined accurately with 4-isopropylnioxime.

LITERATURE CITED

(1) Ferguson, R. C., Voter, R. C., and Banks, C. V., *Mikrochemie ver. Mikrochim. Acta* **38**, 11 (1951).
(2) Hooker, D. T., and Banks, C. V., unpublished work.
(3) Voter, R. C., "Inner-Complex Compounds of Alicyclic *vic*-Dioximes," Ph.D. thesis, Iowa State College, Ames, Iowa, 1951.
(4) Voter, R. C., and Banks, C. V., ANAL. CHEM. **21**, 1320 (1949).
(5) Voter, R. C., Banks, C. V., and Diehl, H., *Ibid.*, **20**, 458 (1948).
(6) *Ibid.*, p. 652.
(7) Wallach, O., and Weissenborn, A., *Ann. Chem. Justus Liebigs* **437**, 172 (1924).

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3-Hydroxyl-1,3-diphenyltriazine as Reagent for Palladium

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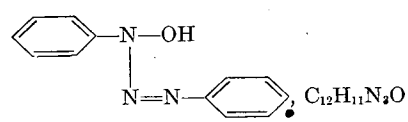
3-Hydroxyl-1,3-diphenyltriazene forms a directly weighable complex with palladium and can be used for the estimation of palladium and also for its separation from various metals including platinum. It is superior to dimethylglyoxime.

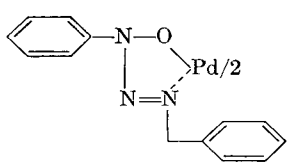
CUPFERRON, the ammonium salt of nitrosophenylhydroxylamine, has been used extensively for the estimation and separation of various inorganic elements. Cupferron, as well as its complexes, is, however, thermally unstable and unsuitable for direct weighing. The reagent is also photosensitive and undergoes gradual deterioration even on careful storage. It is evident that there is considerable scope for improvement in the structural features of such a molecule to make it more suitable as an analytical reagent. Shome (*1*) found that benzoylphenylhydroxylamine is a better reagent than cupferron, and along with other substituted phenylhydroxylamine derivatives, he also indicated the possibility of utilizing 3-hydroxyl-1,3-diphenyltriazene (*N*-phenyl-*N*-phenylazohydroxylamine) for the same purpose (*2*).



This compound has now been examined in detail. It has the following useful characteristics: It is stable toward heat, light, and air and can be preserved indefinitely; many of the complexes formed by it are granular, insoluble in water, thermally stable, of definite composition, and suitable for direct weighing; and the reagent is not very soluble in water, but the excess of the reagent can be removed easily either by washing with dilute alcohol or heating the weakly acidic reaction medium, whereby it is completely hydrolyzed to water-soluble products without affecting the complexes formed. Below pH 3 it forms precipitates only with copper(II), palladium(II), iron(II), iron(III), vanadate, vanadium(III), titanium(IV), and molybdate. Except for the first two complexes, the others are decomposed on heating in acidic medium, thus making the reagent highly selective for copper(II) and palladium(II).

In this communication the use of this reagent for estimation of palladium(II) by direct weighing and also its separation from other elements including iron, cobalt, nickel, platinum, etc., are described. Although gold(III), silver, and osmium(IV) do not form complexes with the reagent, they interfere because they are reduced to the metallic state by the reagent. Tin(II) also interferes because it reduces palladium(II) to the metallic state. In the separation of palladium(II) and platinum(IV) in hot solution, if the quantity of platinum(IV) is more than two times than that of palladium(II), the results are somewhat higher. This is possibly due to the reduction of platinum(IV), followed by subsequent coprecipitation either as metal, or as complex, or as both. However, this defect can be completely eliminated by a second precipitation, or better by carrying out the initial separation in the cold when the separation of one part of palladium(II) from about 10 parts of platinum(IV) becomes possible in one stage through direct weighing. Dimethylglyoxime is the conventional reagent for the estimation of palladium(II) and also for its separation from various elements. However, the present reagent is superior to dimethylglyoxime in many respects. Its palladium complex is more stable toward heat and acid. It is granular, not voluminous like the dimethylglyoxime complex, and can be filtered quickly in the hot state. Compared to dimethylglyoxime, it can also be used with better advantage for the separation of palladium from platinum.

The palladium complex corresponds to the formula, $(C_{12}H_{10}N_3O)_2Pd$. It is best represented as a possible chelate as shown below:



REAGENTS AND APPARATUS

3-Hydroxyl-1,3-diphenyltriazene is a new organic compound whose preparation has not been described in the literature. It was prepared as follows.

Freshly prepared crystalline phenylhydroxylamine (22 grams) was dissolved in warm water (700 ml.), and the solution stirred mechanically with sufficient quantity of crushed ice to bring the temperature to 0° C. A solution of benzenediazonium chloride prepared from 18.6 ml. of aniline, 60 ml. of concentrated hydrochloric acid, and 13.8 grams of sodium nitrite was then slowly added with mechanical stirring to this solution. Small portions of a solution of sodium acetate (100 grams in 300 ml. of water) were occasionally added to the reaction mixture to prevent it from becoming too acidic. After the addition of diazonium salt was complete, the remaining portion of sodium acetate was added and the reaction mixture stirred for another 5 minutes, the temperature during the entire course of reaction being kept at about 0° C. The granular, cream-yellow precipitate of 3-hydroxyl-1,3-diphenyltriazene was then filtered off under suction, washed thoroughly with water, and crystallized two times from alcohol. It was obtained as pale yellow crystals, giving a yield of about 20 grams and having a melting point of 119.5–120° C.

Analysis: found: C, 67.22; H, 4.9; N, 20.16%; $C_{12}H_{11}N_3O$ requires: C, 67.6; H, 5.1; N, 19.9%.

Standard Palladium Solution. One gram of palladium chloride was dissolved in 6 ml. of concentrated hydrochloric acid (analytical reagent) and the solution diluted to 100 ml.; the content of palladium was estimated by using dimethylglyoxime.

Reagent Solution. A solution of the reagent 1% w./v. in 95% ethyl alcohol was used.

Metal Ion Solutions. Reagent grade soluble salts (usually nitrates or acetates, 1% solution) of various metals were used for testing the specificity of the reagent against different ions and also for quantitative separations. Chlorides and sulfates were also used on certain occasions to suit the requirements. Platinic chloride, ceric ammonium sulfate, ferrous ammonium sulfate, and ferric alum were used as the source of platinum(IV), cerium(IV), iron(II), and iron(III), respectively. The following ions were tested: cobalt(II), nickel(II), zinc(II), cadmium(II), antimony(III), manganese(II), copper(II), lead(II), iron(II), iron(III), mercury(II), tin(II), tin(IV), silver, gold(III), osmium(IV), titanium(IV), palladium(II), vanadium(III), aluminum(III), beryllium(II), arsenic(III), thorium(IV), germanium(IV), platinum(IV), cerium(IV), uranium(IV), uranyl(II), tungstate(II), phosphate(III), molybdate(II), vanadate(II), magnesium(II), calcium(II), strontium(II), barium(II), and alkali metals.

Buffering Solutions. Normal hydrochloric acid, 10% w./v. sodium acetate, and sodium potassium tartrate solutions were used for the adjustment of pH. On a few specially required occasions glacial acetic acid and normal sulfuric acid were used instead of hydrochloric acid.

Apparatus. All pH measurements were carried out using a Beckman pH meter Model H-2. A No. 3 sintered crucible was used for collecting precipitates, No. 40 ashless filter-paper for general filtration, and silica crucibles were used for ignition. A Beckman Model DU quartz spectrophotometer was used for determining the spectra of the reagent and the complex.

PROPERTIES OF PALLADIUM COMPLEX OF 3-HYDROXYL-1,3-DIPHENYLTRIAZENE

The palladium complex is a yellowish brown substance, very soluble in chloroform and benzene, fairly soluble in ether and acetone, and only slightly soluble in alcohol. It can be crystallized from acetone in the form of silky needles having a melting point of 252° C. It does not show any sign of decomposition before melting and can be safely heated at 120° to 130° C. to a constant weight in about 45 minutes. Microanalysis of the complex, carried under standard conditions, showed N, 15.7%; $(C_{12}H_{10}N_3O)_2Pd$ calculated: N, 15.82%.

The absorption spectra of both the reagent and the palladium complex have been determined in alcoholic solution.

$\lambda_{max.}$ for reagent 239, 284, 350 mμ; Log E = 4.096, 3.668, 4.276
$\lambda_{max.}$ for complex 225, 255, 413 mμ; Log E = 4.5847, 4.5847, 4.3835, respectively

Effect of pH on Precipitation of Palladium(II). With a view to ascertaining the versatility of this reagent, precipitation of palladium(II) under various pH conditions with varying quantities of the reagent was carried out. Results were checked gravimetrically, as well as by spot tests, using dimethylglyoxime and stannous chloride. The palladium(II) was completely precipitated between pH 1.6 and 8, with only a slight excess of the reagent. Most of the estimations and separations were carried out, however, between pH 2 and 2.5 as it increased the specificity of the reagent and facilitated the removal of the excess of the reagent by hydrolysis. The precipitate formed under these conditions was granular and was filtered easily.

EXPERIMENTAL PROCEDURE

A number of palladium determinations using varying quantities of palladium were carried out according to the following procedure. The final pH measurements were carried out with a glass electrode on the filtrate after removal of the complex.

Procedure for Palladium (II). A solution of palladium chloride containing 10 to 25 mg. of the metal was diluted to about 150 ml.

and 1.5 to 3 ml. of 10% w./v. sodium potassium tartrate or sodium acetate added to it. The requisite amount of normal hydrochloric acid (about 2 ml.) was then added to bring the pH between 2 and 3. pH adjustment could actually be done by adding mineral acid alone, but incorporation of a small amount of sodium acetate or sodium potassium tartrate was found to be helpful in stabilizing it. In most of the separations sodium potassium tartrate was used, as it was found to give slightly more concordant results. To this solution, which may also contain varying quantities of foreign ions, 1% w./v. alcoholic solution of the reagent was added in excess (20 to 25%) with stirring. The palladium complex separated out as a light green precipitate. The suspension was heated on a boiling water bath with occasional stirring for 25 to 40 minutes until the complex became granular and its color gradually changed through yellowish green to yellowish brown. The medium also became clear and free from colloidal suspension. The complex was then filtered either immediately or after 5 minutes through a weighed sintered-glass crucible No. 3, washed with hot water a few times, dried to a constant weight at 120° to 125° C. for 30 to 45 minutes, and weighed.

The weight of the complex multiplied by 0.2009 gave the weight of palladium. In the separation of palladium(II) from iron(II) or iron(III), digestion after precipitation should be carried out for at least 45 minutes, as the bluish black iron complex, which is also partly coprecipitated with palladium, is thereby decomposed and completely goes into solution leaving the palladium complex in a pure state. In the separation from both cerium and zirconium no sodium acetate or sodium potassium tartrate was used. pH was adjusted by dilution with water alone, because prolonged heating in presence of a buffering agent showed turbidity with cerium and zirconium salts giving high results. In the case of cerium, a small amount of ammonium sulfate was also added to keep the cerium salt in solution in the form of double sulfate. The results are given in Table I.

Table I. Determination of Palladium and Its Separation from Other Elements

	Pd Taken, G.	Foreign Ion, G.	Pd Complex, G.	Pd Found, G.	Error, G.
1	0.02700		0.1344	0.02700	0.00000
2	0.01080		0.0537	0.01078	−0.00002
3	0.01080		0.0538	0.01080	0.00000
4	0.01080		0.0537	0.01078	−0.00002
5	0.01754		0.0874	0.01755	+0.00001
6	0.01754		0.0875	0.01757	+0.00003
7	0.01754		0.0875	0.01757	+0.00003
8	0.01080	0.02 Ni(II)	0.0537	0.01078	−0.00002
9	0.01080	0.02 Co(II)	0.0540	0.01084	+0.00004
10	0.01080	0.02 Zn(II)	0.0537	0.01078	−0.00002
11	0.01080	0.02 Al(III)	0.0539	0.01082	+0.00002
12	0.01080	0.02 Cr(III)	0.0538	0.01080	0.00000
13	0.01080	0.02 Cd(II)	0.0537	0.01078	−0.00002
14	0.01080	0.02 Mn(II)	0.0536	0.01076	−0.00004
15	0.01080	0.02 Sb(III)	0.0539	0.01082	+0.00002
16	0.01080	0.02 Bi(III)	0.0535	0.01074	−0.00006
17	0.01080	0.02 As(III)	0.0538	0.01080	0.00000
18	0.01080	0.02 Be(II)	0.0536	0.01076	−0.00004
19	0.01080	0.02 UO_2(II)	0.0539	0.01082	+0.00002
20	0.01080	0.02 Fe(III)	0.0540	0.01084	+0.00004
21	0.01080	0.02 Fe(II)	0.0539	0.01082	+0.00002
22	0.01169	0.02 Zr(IV)	0.0584	0.01172	+0.00003
23	0.01169	0.02 Ce(IV)	0.0580	0.01164	−0.00005
24	0.01169	0.50 nitrate	0.0583	0.01170	+0.00001

No actual separation of palladium from alkali, alkaline earth metals, and lead was carried out. In the case of lead, insoluble chloride of the metal is precipitated in the presence of palladium chloride. However, as none of these elements form any precipitate with the reagent, their separation from palladium appears to be possible under suitable conditions.

Separation of Palladium from Platinum. Results of the separation of palladium from platinum are recorded in Table II. The procedure for the separation was the same as described above if the quantity of platinum was not more than two times that of palladium; the only difference was that the period of digestion on the water bath was restricted to 15 to 20 minutes (estimations 1 to 8, Table II). When the quantity of platinum was more, the following two modified methods were used, and excellent results were obtained even when the platinum content of the mixture was about 10 times that of palladium.

Modified Procedure I. The palladium, as described earlier, was precipitated in the cold at pH 2.5 by addition of 20% excess of the reagent. After 5 minutes the dirty green palladium complex was filtered through a sintered-glass crucible (No. 3) and washed five to six times with cold dilute hydrochloric acid solution (1%, v./v.) for complete removal of platinum. From the combined filtrates platinum could be estimated, if desired, in the conventional way.

Table II. Determination of Palladium in Presence of Platinum

	Pd Taken, G.	Pt Taken, G.	Pd Complex, G.	Pd Found, G.	Error, G.	Pt Found, G.	Error, G.
1	0.01080	0.010	0.0537	0.01078	−0.00002		
2	0.01080	0.010	0.0539	0.01082	+0.00002		
3	0.01080	0.010	0.0538	0.01080	0.00000		
4	0.01080	0.015	0.0536	0.01076	−0.00004		
5	0.01080	0.020	0.0539	0.01082	+0.00002		
6	0.01080	0.020	0.0537	0.01078	−0.00002		
7	0.01169	0.020	0.0583	0.01170	+0.00001		
8	0.01169	0.020	0.0582	0.01168	−0.00001		
9	0.01221	0.0410	0.0609	0.01223	+0.00002	0.0414	+0.0004
10	0.01221	0.0410	0.0608	0.01221	0.00000	0.0408	−0.0002
11	0.01221	0.1023	0.0605	0.01215	−0.00006	0.1017	−0.0006
12	0.01221	0.0878	0.0610	0.01225	+0.00004	0.0872	−0.0006
13	0.01221	0.0878	0.0611	0.01227	+0.00006	0.0880	+0.0002
14	0.01221	0.1250	0.0609	0.01223	+0.00002	0.1245	−0.0005
15	0.01169	0.030	0.0580	0.01164	−0.00005		
16	0.01169	0.030	0.0579	0.01163	−0.00007		
17	0.01169	0.050	0.0582	0.01168	−0.00001		
18	0.01169	0.100	0.0585	0.01174	+0.00005		

The palladium complex, which still contained an excess of the reagent, was transferred from the sintered crucible to a beaker with a jet of water (50 to 70 ml.); 2 ml. of 2*N* hydrochloric acid was added and the mixture heated on the water bath for 1 hour to destroy the excess of the reagent. The palladium complex changed color as previously, from dirty green to yellowish brown, and became granular. It was filtered through the same sintered crucible, washed well with hot water, dried at 120° C., and weighed. The results obtained are shown in Table II, Nos. 9 to 14.

Modified Procedure II. According to this, the palladium complex was precipitated as usual by addition of about 20% excess of the reagent, heated on the water bath for 15 to 20 minutes, filtered on an ashless filter paper, washed thoroughly with hot water, dried, and carefully ignited with usual precautions necessary to avoid any loss of palladium. The residue was then dissolved by heating in 4 ml. of concentrated sulfuric acid and 1 ml. of concentrated nitric acid, until copious fumes of sulfuric acid began to evolve. This ensured the complete removal of nitric acid. The residue was then carefully transferred to a beaker by washing with water, and the volume was made up to 200 ml. and neutralized with analytical reagent sodium carbonate. Finally, hydrochloric acid (5 to 7 ml. of normal hydrochloric acid) was added to bring the pH between 2 and 3. Palladium was then precipitated in the usual way by the addition of the reagent and estimated by direct weighing.

The results are shown in Table II (estimations 15 to 18). For convenience and speed the modified procedure I is preferred.

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LITERATURE CITED

(1) Shome, S. C., *Analyst* **75**, 27 (1950).
(2) Shome, S. C., *Current Sci. (India)* **13**, 257 (1944).

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Amperometric Determination of Vanadium

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An amperometric procedure has been developed for the determination of vanadium in titanium-base alloys and alloy steels. The procedure is simple, rapid, and accurate within at least 1% in the presence of titanium, iron, chromium, manganese, and small amounts of molybdenum and tungsten. In general, molybdenum and tungsten concentrations equivalent to that of the vanadium are permissible, but low results will follow the removal of more than about 2% of tungsten which separates as the oxide.

THE technique of Lang and Kurtz (*2*), as studied by Walden, Hammett, and Edmonds (*3*), for the titrimetric determination of vanadium involves a preliminary valence-state adjustment of the vanadium based on the following sequence of reactions (unbalanced), all of which proceed quantitatively in a 5% sulfuric acid medium:

$$V^{+4} + \text{excess } KMnO_4 \rightarrow V^{+5} + Mn^{++}$$

$$\text{Excess } KMnO_4 + \text{excess } KNO_2 \rightarrow Mn^{++} + KNO_3$$

$$\text{Excess } KNO_2 + \text{excess urea} \rightarrow N_2$$

The solution then contains quinquevalent vanadium, manganous ion, and urea. The use of cold permanganate solution in moderate excess avoids the oxidation and interference of elements such as chromium. Manganous ion and urea are not harmful in the later steps of the procedure. Walden, Hammett, and Edmonds (*3*) then employed a 30% sulfuric acid medium for the titrimetric step with standard ferrous sulfate solution and ferrous–*o*-phenanthroline (1,10-phenanthroline) indicator:

$$Fe^{++} + V^{+5} \rightarrow Fe^{+++} + V^{+4}$$

The high acidity is not essential to the reaction cited, but merely serves to place the indicator transition at the proper potential relative to the V^{+5}/V^{+4} and Fe^{+++}/Fe^{++} couples.

Willard and Young (*4*) employed similar valence-state adjustments, using hydrofluoric acid in the presence of tungsten.

An amperometric determination of vanadium based on this procedure (but in 5% acid throughout) seemed to be feasible. A rotating platinum electrode was employed, since the reduction of vanadium(V) on mercury proceeds rapidly. Furman, Reilley, and Cooke (*1*) have described a coulometric technique for the microdetermination of vanadium.

EXPERIMENTAL

Apparatus. The titrations were carried out in one arm of an H-type polarographic cell using the Sargent Ampot in conjunction with a rotating platinum-indicator electrode and a reference saturated calomel electrode. The two arms of the titration cell were separated by a glass frit. Stirring was effected by the rotating platinum electrode. No purging of the system by nitrogen gas was needed in this determination. The electrode consisted of platinum wire sealed in a glass tube with approximately 2 mm. of platinum wire protruding from the tube. Electrical contact was made to the electrode through a mercury pool in the glass tube. A 10-ml. buret graduated to 0.05 ml. with an offset tip was used in all titrations, whereas a 25-ml. pipet was used to measure the amount of solution to be titrated.

Chemicals and Reagents. SULFURIC ACID. Reagent grade (96%) acid was diluted to give a 5% (about 1*M*) solution.

o-PHENANTHROLINE–FERROUS COMPLEX (ferroin). This was used as a 0.025*M* solution.

All other chemicals were reagent grade materials and were used without further purification. Potassium permanganate, 1% by weight, and potassium nitrite, 2% by weight, solutions were prepared as needed.

FERROUS IRON STOCK SOLUTION. This solution was approximately 0.1*N* in iron(II) and 5% in sulfuric acid. It was restandardized every second day against a standard dichromate solution.

AMMONIUM VANADATE. Approximately 11.7 grams of ammonium vanadate was dissolved in 5% sulfuric acid to make 1 liter of solution. This was standardized against the iron(II) solution using ferroin as indicator. The concentration was found not to change with time.

VANADIUM SAMPLES. Titanium-base alloys, samples WA-14 and WA-15, containing a known percentage of vanadium were obtained from the Watertown Arsenal, Watertown, Mass. These materials were available as a part of a cooperative analysis-study project. Bureau of Standards and other materials were also used.

Development of Procedure. Early trials indicated that a procedure might be based on titration at a fixed potential setting of about +0.10 to +0.30 volt *vs.* S.C.E. The growth of the ferric reduction wave during the titration was, presumably, being followed. Later results by another worker failed to confirm the reliability of this procedure. The polarographic waves of the systems involved are shown in Figure 1. It is evident from the figure that the ferric to ferrous reduction wave is too close to the vanadium system for resolution.

It appeared, however, that a usable method might be based on a more positive potential setting, measuring the rate of appearance of the anodic ferrous oxidation wave beyond the equivalence point. This was tested by titrating 0.0100*N* vanadium(V) solution with 0.1041*N* iron(II) solution while varying the applied voltage from +0.40 to +0.70 volt by steps of 0.05 volt in a series of seven titrations. The results given in Table I were obtained.

Table I. Amperometric Titration of Vanadium(V) Solution with Iron(II) Solution at Different Applied Potential Values

Applied Potential *vs.* S.C.E., Volt	Iron(II) Vol. of Soln., Ml.	
	Calcd.	Used
+0.40	2.38	1.8
+0.45	2.38	1.4
+0.50	2.38	1.3
+0.55	2.38	2.13
+0.60	2.38	2.37
+0.65	2.38	2.37
+0.70	2.38	2.33

Those potential settings more positive than about 0.55 to 0.60 volt appear to be satisfactory with the concentrations used. A figure of +0.65 volt was tentatively adopted as safe for all reasonable concentrations of vanadium. A more positive setting than required is undesirable, of course, as it would increase the possibility of interferences. A series of 10^{-2} to $10^{-4}N$ vanadium solutions was run at +0.65 volt with the very satisfactory results shown in Table II.

In general, a 25-ml. sample of ammonium vanadate solution of suitable concentration was prepared from the stock vanadium solution by dilution. This was placed in the titration portion of the H-cell and rotation of the platinum electrode was begun. The titrant, a standardized iron(II) solution, which was approximately 10 times the normality of the vanadate solution in question, was added in 0.5-ml. increments until the end point was reached. Addition of titrant was continued past the end point in 0.5-ml. portions. The current was read 0.5 to 1.0 minute after each addition at a potential of 0.65 volt *vs.* S.C.E. The points obtained in this manner yielded a pair of straight lines that inter-

sected at the equivalence point when plotted in the conventional way (Figure 2). A very desirable time-saving feature of the procedure, based on operating at this potential, is the fact that oxygen causes no interference and neither the solution being titrated nor the titrant need to be deaerated.

Interferences. There is obviously no reason to expect any interference by the many elements (such as copper, magnesium, aluminum, etc.) which cannot be oxidized to higher states at +0.65 volt and which are not reduced at so positive a potential. Appreciable amounts of manganese are introduced during the initial oxidation-state adjustment and remain as manganous ion after the nitrite addition. Manganese initially present would be in this same state. The ferrous titrant contains ferric ion. Obviously, then, iron and manganese do not interfere.

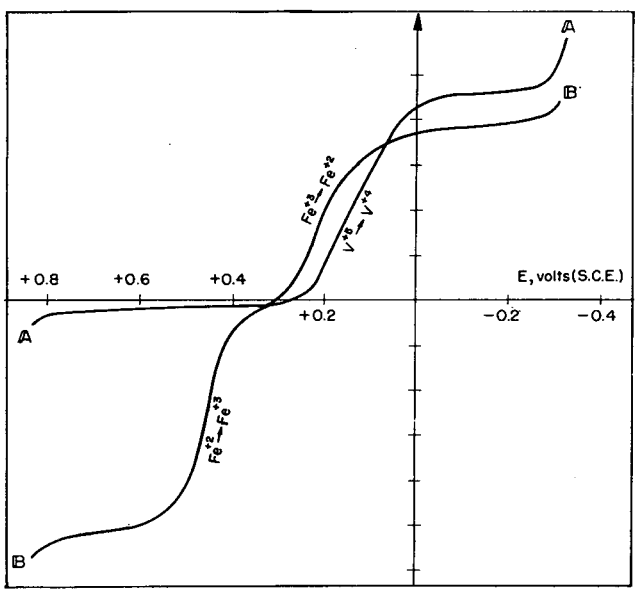

Figure 1. Polarographic waves

A. Reduction of vanadium(V) solution
B. Oxidation-reduction of iron(II) and iron(III) solution

Equivalent amounts of chromium, molybdenum, and tungsten were added to 0.0100*N* vanadium solutions, which were then analyzed amperometrically. The solutions containing tungsten and molybdenum had indistinct end points because of the gradual "trailing off" of the last part of the titration plot (an additional change in slope and some curvature appears about 1.5 to 2 ml. beyond the equivalence point). Chromium showed very little interference. Vanadium can be determined in the presence of equivalent amounts of molybdenum and tungsten if the points immediately following the end point are taken sufficiently close together to determine a straight line; there is no trouble in detecting the end point when these elements are present in small proportions. Typical titration results are given in Table III.

Analytical Procedure. Dissolve a 0.2- (vanadium content exceeding 5%) to 2.0-gram (vanadium content less than 0.5%) sample in either A or B.

A. Ten (0.2-gram sample) to 50 ml. (2-gram sample) of 50% sulfuric acid contained in a 250-ml. borosilicate glass beaker.

B. Small quantities of 48% hydrofluoric acid as needed, contained in a suitably covered platinum dish; add nitric acid dropwise, as required, to hasten dissolution. After solution is complete, add a measured volume 10 to 50 ml., as in step A, of 50% sulfuric acid. Then evaporate the mixture to light fumes of sulfuric acid (sulfur trioxide fumes).

Add 1% permanganate solution dropwise to the cooled, diluted

Table II. Amperometric Titration of Vanadium(V) Solution with Iron(II) Solution at Applied Potential of +0.65 Volt *vs.* S.C.E.

V^{+5} Soln. Taken		Iron(II) Soln. Required		
			Volume, Ml.	
Vol., ml.	Concn., *N*	Concn., *N*	Calcd.	Used
25.0	0.0101	0.0950	2.52	2.50
25.0	0.0100	0.1031	2.44	2.40
25.0	0.0050	0.0520	2.41	2.45
25.0	0.0010	0.01 4	2.41	2.48
25.0	0.0005	0.0052	2.41	2.48
25.0	0.0001	0.0010	2.41	2.40

Table III. Amperometric Titration of Vanadium in Presence of Chromium, Molybdenum, or Tungsten

Vanadium Taken		Additive and Concn.	Iron(II) Soln. Required		
				Volume, Ml.	
Vol., ml.	Concn., *N*		Concn., *N*	Calcd.	Used
25.0	0.0100	0.01*N* Cr^{+++}	0.1020	2.34	2.34
	0.0998	0.1*N* Mo^{+6}	0.9340	2.52	2.54
	0.0100	0.01*N* Mo^{+6}	0.1024	2.48	2.44
	0.00998	0.01*N* Mo^{+6}	0.0934	2.52	2.50
	0.0100	0.01*N* W^{+6}	0.0980	2.55	2.48

(final volume of 25 to 40 ml.) solution until a permanent pink color persists. Then add 2% potassium nitrite solution dropwise until the excess permanganate is destroyed—i.e., the solution is decolorized. Finally, add about 3 to 4 grams of urea.

Depending on the expected vanadium content (and sample size), dilute the solution to any convenient exact volume from 50 to 500 ml., such as to give a vanadium concentration of at least $5 \times 10^{-4}N$ and not more than $10^{-2}N$. The total sulfuric acid used in steps A or B should be such as to give a 5 to 10% concentration of sulfuric acid in the final solution.

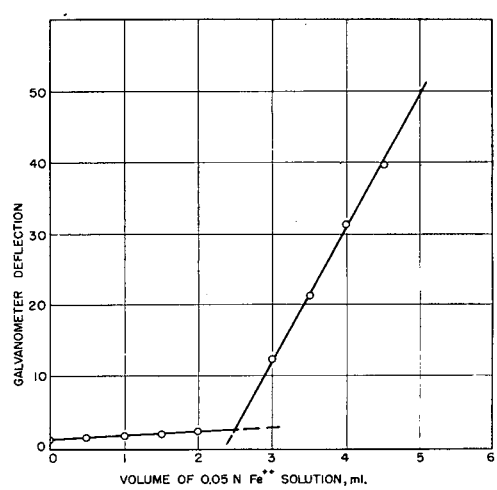

Figure 2. Amperometric titration of 25 ml. of 5m*M* vanadium(V) at 0.65 volt (S.C.E.)

Transfer a 25- or 50-ml. aliquot to a suitable amperometric titration cell. Immerse in the cell a rotating platinum electrode and a suitable connection (salt-bridge type) to a saturated calomel electrode (S.C.E.). The junction of the connection to the cell and the cell itself must be free of leakage, as dilution of the cell solution or the reference electrode could produce serious errors. Set the electrolyzing potential at the rotating platinum microelectrode at +0.65 volt. Read the current on any suitable shunt and galvanometer, or microammeter assembly.

Add from a 5- or 10-ml. microburet standard ferrous sulfate solution, whose normality should be approximately 10 times that of the vanadium. Take volume (of titrant) and (corresponding) current readings at about 0.25- to 0.50-ml. intervals for 1.5 to 2.0 ml. before and beyond the equivalence point. (At least four to

five good points are needed on either side of the equivalence point. These should extend at least 1.5 ml. on either side; points closer than 0.1 ml. should be avoided.) Correct the observed current readings, G, for dilution by the titrant through the relation

$$G_{corr.} = G \frac{V_o + V_x}{V_o}$$

where V_o is the initial volume of the vanadium solution being titrated and V_x is the volume of titrant at current reading G. A plot of $G_{corr.}$ *vs.* volume in milliliters of ferrous sulfate defines the equivalence point as the intersection of two straight lines. The data from a typical run are shown in Figure 2.

Check runs are readily made on the same sample by taking fresh aliquots for titration.

Table IV. Determination of Vanadium in Titanium Samples by Amperometric Titration

Designation Wt., G.	Solvent	Iron(II) Soln. Required: Concn., *N*	Iron(II) Soln. Required: Vol., ml.	Vanadium Content, %: Reported	Vanadium Content, %: Found
WA-15 1.005	50% H_2SO_4	0.0493	2.48	2.53	2.50
WA-15 1.008	HF + H_2SO_4 to fumes	0.0493	2.50	2.53	2.49
WA-14 1.016	50% H_2SO_4	0.1005	1.30	2.64	2.62
WA-14 1.003	HF + H_2SO_4 to fumes	0.0503	2.55	2.64	2.64

RESULTS

The data shown in Table IV are representative of the results obtained on the two available titanium-base alloys.

The results on a variety of alloys, containing significant amounts of chromium, manganese, tungsten, titanium, molybdenum, cobalt, and other constituents, are shown in Table V.

Table V. Amperometric Determination of Vanadium in Ferrous Alloys

Sample Designation	Vanadium Content, %: Reported	Vanadium Content, %: Found
NBS No. 111 steel	0.003	0.005
NBS No. 116a ferrotitanium	0.33	0.343
Brit. Chem. Std. "V" steel	0.273	0.274, 0.269
NBS No. 134 steel	1.13	1.12, 1.11[a]
NBS No. 153 steel	2.04	2.00[a]

[a] Separated WO_3 filtered off and acid-washed.

It is evident that the method, which is rapid and direct, is capable of giving good results over a wide range of vanadium concentrations, and in the presence of any of the common alloying constituents. The amperometric end-point determination is more objective in character than are comparable indicator techniques, and is especially advantageous at low concentrations where indicator blanks are significant. Its success in the presence of equal amounts of tungsten is especially noteworthy, but it should be anticipated that low results would certainly follow the removal (by filtration of separated tungstic oxide) of tungsten in excess of about 2%.

LITERATURE CITED

(1) Furman, N. H., Reilley, C. N., and Cooke, W. D., ANAL. CHEM. **23**, 1665 (1951).
(2) Lang, R., and Kurtz, F., *Z. anal. Chem.* **86**, 288 (1931).
(3) Walden, G. H., Hammett, L. P., and Edmonds, S. M., *J. Am. Chem. Soc.* **56**, 57 (1934).
(4) Willard, H. H., and Young, P., IND. ENG. CHEM., ANAL. ED. **4**, 187 (1932); **5**, 154 (1933); **6**, 48 (1934).

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Determination of Cure and Analysis of Cured Epoxy Resins

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The degree of cure of thermosetting epoxy resins is determined from the extent of the chemical conversion and the extent of cross linking. The chemical conversion is calculated from the residual epoxy content. Two methods for determination of epoxy groups in insoluble resins are presented, one based on infrared spectroscopy, the other on chemical reaction of a suspension of the resin in a swelling agent. The estimation of the cross linking of cured resins is based on their resistance to deformation at elevated temperatures and to swelling in organic solvents. In the first case, a hot-point hardness tester is used; in the second, the finely powdered resin is exposed to the vapors of a swelling agent until equilibrium has been reached. Data obtained on experimental and commercial epoxy resins are presented.

THE advent of the epoxy resins has brought with it a number of analytical problems, relating not so much to the epoxides in the esterified form—i.e., after combination with drying fatty acids—as to the epoxides that are converted into a hard, insoluble, and infusible form by the effect of chemical curing agents and by a reaction commonly called "cure."

The physical and mechanical properties of a cured epoxy resin are to a large degree dependent on the type and amount of curing agent as well as on the cure time and temperature of cure. A systematic study of these factors appeared desirable, especially an evaluation of the degree of cure as a function of the cure conditions. The term "cure" is a rather loose description of the reaction that converts a thermosetting resin from the soluble, fusible into the insoluble, infusible state and does not lend itself to numerical evaluation. It was necessary to define this term more precisely for the purpose of quantitative studies.

Cure may be subdivided into two components. One, called conversion, describes the chemical reaction during the cure and expresses it as disappearance of reactive groups. In the case of epoxy resins, these are the epoxy groups, and when, for example, 80% of the initially present epoxy groups have been used up to form a polymer, the conversion would amount to 80%. The other component is related to the three-dimensional structure or network that appears during the cure, called cross linking. The degree of cross linking is dependent on the progress of the chemical reaction—i.e., the conversion—but it is also related (*11*) to the functionality of the chemical compounds entering the reaction.

The knowledge of both conversion and cross linking appears to give a complete description of the cure of a thermosetting resin and one that lends itself to quantitative evaluation. The experimental material in this article is confined to one type of epoxy resins—namely, the condensation polymers of epichloro-

Table I. Determination of Conversion of Cured Epon 828

Curing Agent, Parts/100 Parts Epon	Cure Conditions: Time, hours	Cure Conditions: Temp., °C.	Spectroscopic Method: Epoxy value, equiv./100 g.	Spectroscopic Method: Conversion, %	Chemical Method: Epoxy value, equiv./100 g.	Chemical Method: Conversion, %
Piperidine[a], 5	24	65	0.103	78.8	0.096	80.3
	+ 4[b]	100	0.085	82.5	0.087	82.1
	+ 1[b]	150	0.074	84.8	0.074	84.8
	+ 0.5[b]	200	0.067	86.2	0.059	87.9
Tris(dimethylaminomethyl)phenol, 4	24	65	0.099	79.8	0.106	78.4
	+ 4[b]	100	0.072	85.3	0.072	85.3
	+ 1[b]	150	0.049	90.0	0.024	95.1
	+ 0.5[b]	200	0.045	90.8	0.016	96.7
m-Phenylenediamine[a], 12.5	24	65	0.124[c]	69.8	0.12	70.6
	+ 4[b]	100	0.117	71.5	0.108	73.5
	+ 1[b]	150	0.090	78.0	0.105	74.3
	+ 0.5[b]	200	0.083	79.7	0.095	76.8

[a] Pure compound.
[b] Cures in addition to cures indicated in line or lines above.
[c] Value determined by rock salt cell method and used as calibration standard.

hydrin and bisphenol A that are known commercially as Epon (Shell Chemical Corp.) resins—and to one class of curing agents, the organic amines.

DETERMINATION OF CONVERSION

The conversion, in per cent, of a cured epoxy resin is calculated as

$$\frac{100 \times (\text{epoxy value of uncured resin} - \text{epoxy value of cured resin})}{\text{epoxy value of uncured resin}}$$

The epoxy value of the uncured resin is easily determined by known methods (*10, 12*). However, the determination of epoxy values in cured epoxy resins presented problems in view of the insolubility of these materials. No similar material of precisely known composition was available to serve as a standard in the development of a new method. Under these circumstances, efforts to develop a suitable method were carried out in two different directions, hoping that it would be possible to corroborate the results of one method by those of the other. The same resins were used for each method.

Determination of Epoxy Content of Cured Epon Resins. By Infrared Spectroscopy. The infrared analytical method for the determination of epoxy groups in cured Epon resins uses the absorption band at 10.92 microns which the authors assigned, in agreement with findings by Herzberg (*8*) and Patterson (*14*), to a fundamental vibration of the oxirane ring —C—C—. When the epoxy resin is cured, the ring is opened and the 10.92 band disappears (Figure 1). The absorption of the uncured resin is represented by the heavy line. When a curing agent is added and the mixture is heated, cure takes place and the absorption spectrum changes progressively, as indicated, until, at 100% conversion, it assumes the shape indicated by the broken line of Figure 1.

Samples used for recording spectra such as those shown in Figure 1 were prepared as thin films about 0.025 mm. thick between rock salt plates. The curing took place in the rock salt cell, and the spectrum was recorded at various time intervals. Because the film thickness decreased slightly during the first part of the cure, through cell leakage, relative film thicknesses were obtained by measuring the intensity of a band at 6.325 microns due to phenyl group absorption and, accordingly, all 10.92 absorbances were corrected to correspond to a single thickness. To calculate from these spectra the epoxy values of the resins, the absorptivity per epoxy group was determined for the 10.92 band from the spectrum of the uncured resin using the chemically determined epoxy value. The epoxy value of each sample was determined from the absorbance at 10.92 microns divided by the absorptivity. A correction was applied for the film thickness as indicated and a second correction for the presence, at 10.92 microns, of some absorption not caused by epoxides (see Figure 1, broken line).

A cure curve obtained by this method is shown in Figure 2. The method includes the assumption that the absorbance at 10.92 microns per epoxy group is constant regardless of the degree of cure and that there is no deviation from Beer's law. Though these assumptions are reasonable, they could not be confirmed experimentally in view of the unavailability of suitable calibration standards.

The described method is not practical for routine testing, partly because a rock salt cell is sacrificed in each run, and partly because samples are usually submitted in the cured state. For such samples spectroscopic examination was made possible by the use of the pressed plate technique—i.e., by embedding the finely powdered substance in potassium bromide. This tech-

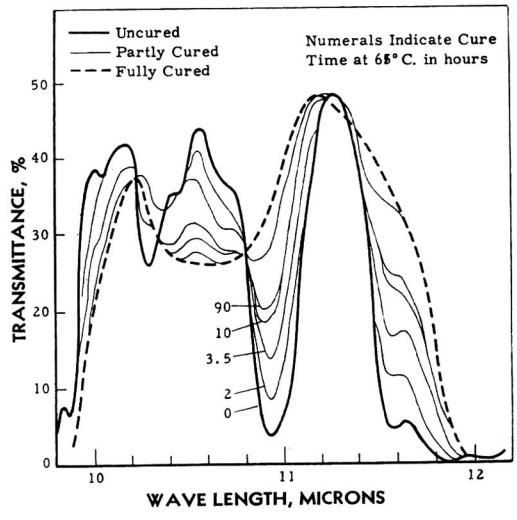

Figure 1. Infrared absorption of epoxy group in Epon resin

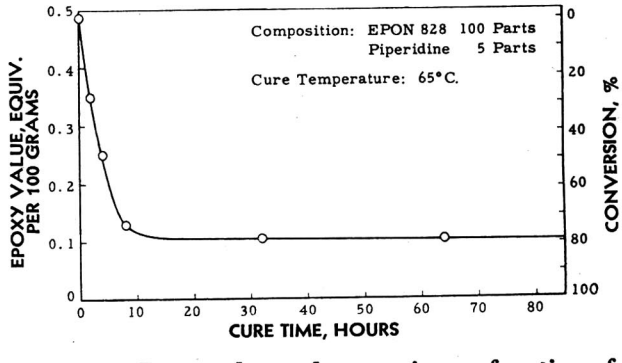

Figure 2. Epoxy value and conversion as function of cure time

nique and its theoretical background have been described (*7, 15, 19, 20*). The comminution of the cured resin to the required particle size of a few microns was carried out by means of a vibratory ball mill (*6*). Potassium bromide proved to be a very suitable carrier for cured Epon resins; the obtained spectra had the same resolution as those obtained on homogeneous samples in rock salt cells.

In the evaluation of the spectra obtained by the pressed plate method, corrections were applied similar to those described for homogeneous samples. In addition, the pressed plate method was calibrated by comparison with the rock salt cell method—i.e., samples of the same composition, cure time, and cure temperature were analyzed by both methods, and the epoxy value obtained by the rock salt cell method was used to calculate the absorptivity to be used in the evaluation of pressed plate data.

Epoxy values and conversions obtained by the pressed plate method on Epon resins of different composition and cure are presented in Table I. The samples were selected to describe the influence of the curing agent as well as the effect of increased cure temperature. The curing agents were: piperidine, a secondary amine, relatively slow; tris(dimethylaminomethyl) phenol, a tritertiary amine, fast and effective; and *m*-phenylenediamine, a diprimary amine, fast and very effective, especially at higher temperatures. The cure schedules were 24 hours at 65° C., 4 hours at 100° C., 1 hour at 150° C., and 0.5 hour at 200° C.; samples cured at the higher temperatures had been subjected to the cure schedules at the lower temperatures. The conversions found for these samples vary from 70 to 91%, and the influence of the cure temperature is pronounced.

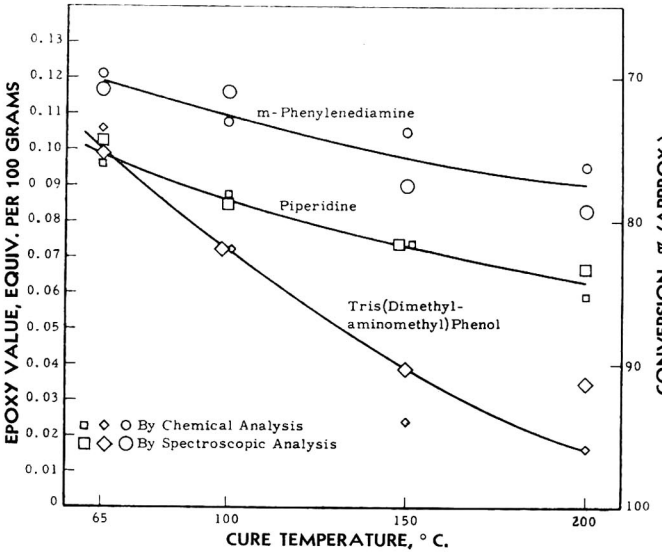

Figure 3. Conversion of Epon 828 upon curing with different curing agents and at different cure temperatures

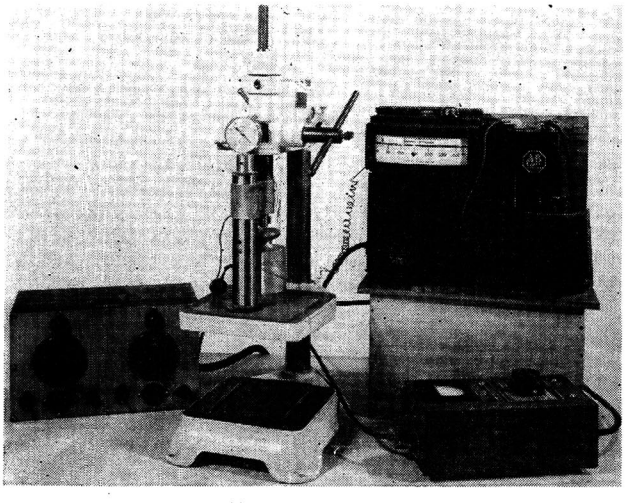

Figure 4. Hot-point hardness tester

By Chemical Analysis. The infrared-spectroscopic method is based on two assumptions: the validity of Beer's law and constant absorbance at 10.92 microns per epoxy group irrespective of the change-over from the liquid to the solid state. Since the correctness of the latter assumption is difficult to prove, an independent method was desired for the determination of residual epoxides in cured epoxy resins. Moreover, there appeared to be a general need for a method for determining functional groups in cured thermosetting resins.

The development of such a method was based on the following considerations. If the resin sample is reduced to a very small particle size and suspended in a liquid which acts as a swelling agent, the molecules of the resin should be accessible by diffusion. More specifically, a solution of an analytical reagent should diffuse into the swollen particle and perform the analytical reaction, and the reaction products should diffuse out of the particle. The time needed for such a reaction decreases with decreasing particle size—i.e., extremely fine particles are required for a rapid reaction.

Chemical reactions in swollen solids are not novel—e.g., the acetylation of cellulose can be carried out in the swollen state without destruction of the fiber structure (*9*), and the acid number of paint films has been determined in a swollen condition (*13*).

Preparation of Samples. As shown by preliminary experiments, the contemplated method is not practical unless the particle size is in the order of a few microns. Thus, the pulverization of the hard resins to micron size is a major part of the problem. A suitable procedure was developed using a Wiley laboratory mill for coarse grinding (to 20-mesh size) and a vibratory ball mill (*6*) for the final pulverizing. This mill reduces coarse powders to very fine powders without formation of much material of intermediate size. A charge of 10 grams and a grinding time of 1 hour are used in most cases. Handling and separation of the fine Epon powders are handicapped by electric charges; however, the difficulties are overcome by using a wet method consisting of the steps of: suspending the ground material in anhydrous alcohol; separating the fine portion from the unchanged coarse material by screening the suspension through a 325-mesh screen; collecting the fine fraction on a Büchner funnel on Whatman No. 50 paper; and vacuum drying the powder at 65° C.

Analytical Procedure. For the determination of epoxy values on the fine powders, a modification of the method of King (*10, 12*) was employed. The substance was suspended in a solution of hydrochloric acid in dioxane and the unused acid titrated after 15 minutes. The reaction appeared to be complete after this time.

Results. The results appear in the last two columns of Table I. Figure 3 serves to compare the spectroscopically obtained values with those determined by chemical analysis. The agreement is fairly good; in some cases identical values were obtained, but in others some discrepancy appears, especially when the epoxy values are very small.

The curves connecting the experimental points obtained for each curing agent are plausible; they show clearly the influence of the cure temperature on the conversion as well as the differences in the activity of the three curing agents. Thus, the presented methods for the determination of the conversion, though still somewhat lacking in precision, are believed to give essentially correct values and can serve as guides for evaluation and comparison of epoxy resins, curing agents, and cure conditions.

ESTIMATION OF CROSS LINKING

The determination of the degree of cure from the amount of chemical conversion is not satisfactory as the sole means of evaluation of thermosetting resins, because the values obtained in this

way do not necessarily agree with the performance of the cured resins. An additional factor to be considered is the extent and character of the three-dimensional network formed during the cure. Though all cured thermosetting resins are three-dimensionally linked, various ways of linking are possible. The molecular weight and functionality of the reacting compounds are likely to have a great influence on the structure formed.

The determination of the degree of cross linking in highly cured thermosetting resins is a problem that has not been fully solved. However, the theoretical treatment by Huggins, Flory, Rehner, and others (*5*), based on methods of statistical thermodynamics, gives a lead on how to correlate the cross linking of polymeric substances with other more easily measurable properties. Two such possibilities have been established; one relates the cross linking to the deformation under load at elevated temperatures, the other to the amount of swelling when immersed in a solvent. Both principles have been utilized to estimate the degree of cross linking in cured epoxy resins.

Determination of Softening Temperature. Known methods for the determination of deformation under load at elevated temperatures include heat distortion tests (*1*), plasticity tests (*2*), and hardness tests with conventional hardness testers on preheated specimens. In the case of cured epoxy resins the situation was complicated by the fact that the temperatures needed to obtain a well measurable deformation are frequently higher than the customary cure temperatures. Thus, an undesirable continuation of the cure may result while the specimen is heated for the test. To avoid this source of error, a testing method was desirable in which the major part of the specimen is kept at room temperature, and heat is applied over a small area and during a limited time only. An arrangement in which a preheated, pointed tool is used to make a measurable indentation into a resin sample appeared to be the ideal solution.

An elaborate instrument of this type has been described (*16*), but is not available commercially; thus, the hot-point indentation tester was developed independently. A photograph of the tester with accessories is given in Figure 4, a drawing of the essential parts in Figure 5. The major features of the instrument are:

The indentation tool is a small steel tip with an included angle of 90°, inserted in a cylindrical copper block.

The copper block is heated electrically to temperatures up to 300° C.

Temperature control is obtained through a copper-constantan thermocouple located very close to the steel tip.

The indenter—i.e., copper block with tip—is loaded by dead weight. The total load is 700 grams.

The hot indenter is lowered smoothly and in precisely perpendicular direction onto the specimen, which must have a plane surface.

After a contact time of 20 seconds, the penetration of the indenter—i.e., the depth of the indentation—is read directly from a dial gage. The range is 0 to 0.025 inch.

The measurements reported were made on 1-mm. thick sheets of cured epoxy resins at temperatures of 25°, 50°, 100°, 150° C., etc., until the depth of indentation reached the limit of the gage. The depth values found were plotted against the temperature at the indentation point. Figure 6 shows a family of curves of the type. From these curves, the temperature at which the indentation reached a depth of 0.0075 inch was determined. This value, called arbitrary softening temperature, is highly typical for a cured resin and was selected as a relative measure for the degree of cross linking.

The choice of the curing agent, as well as the cure temperature, has a strong influence on the softening temperature (Table II, column 4); under favorable circumstances—i.e., when an efficient curing agent and a high cure temperature were employed—values up to 229° C. were obtained.

Determination of Swelling Value. The second of the two principles that are thermodynamically related to the degree of cross linking is the swelling of the cross-linked materials in solvents. To utilize this principle, a method of measuring the swelling of cured resins was developed.

The theory relates the degree of cross linking to the amount of swelling at the swelling equilibrium; thus, one cannot be satisfied with swelling rates, but must continue the swelling procedure until a stationary condition has been established.

Swelling in Liquid Solvents. The conventional method for swelling measurements on cross-linked polymers consists in immersing weighed or measured specimens into suitable solvents and observing the gain of weight or volume over a period of time. This method, tried extensively on samples of cured Epon resins, gave disappointing results. Difficulties were caused by cracking of the specimens or even disintegration into small fragments

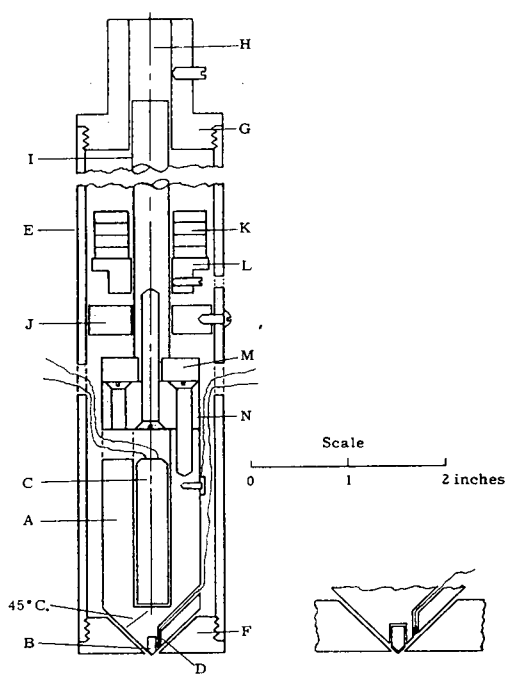

Figure 5. Hot-point hardness tester, indenter assembly

A. Copper cylinder, chrome plated
B. Steel tip
C. Cartridge heater (50 watt)
D. Thermocouple
E. Housing
F. Foot plate
G. Upper bushing and gage holder
H. Insert dial gage (Ames No. 212)
I. Connecting rod
J. Brass bushing
K. Lead disks
L. Loading platform
M, N. Transite blocks

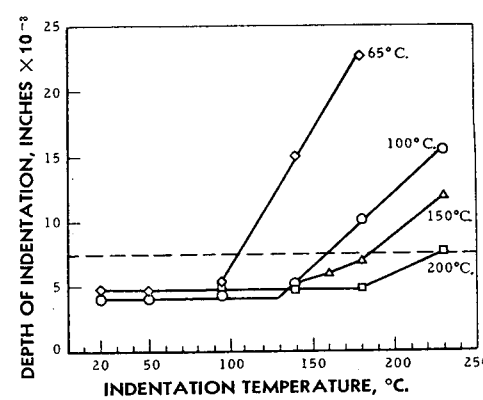

Figure 6. Hot hardness curves of cured Epon 828

Curing agent, *m*-phenylenediamine
Maximum cure temperatures as indicated

while the swelling was in progress (*17*, *18*). Even where the cracking was not very obvious, the swelling went through a maximum instead of approaching an equilibrium. Similar observations have been reported and discussed (*3*, *4*). Since the method did not give equilibrium values on cured Epon resins, it was considered unsuitable for the present purposes.

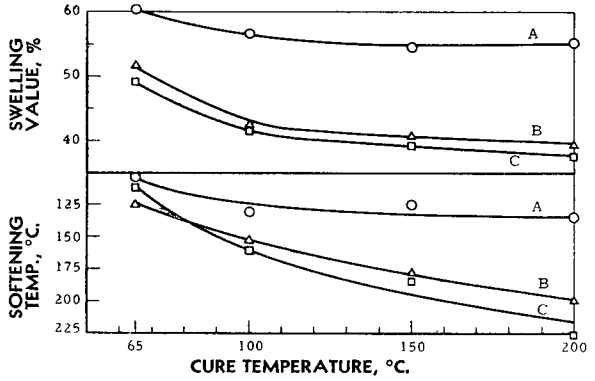

Figure 7. Comparison of swelling values and softening temperatures of cured Epon 828

A. Curing agent, piperidine
B. Curing agent, tris(dimethylaminomethyl)phenol
C. Curing agent, *m*-phenylenediamine

Swelling in Solvent Vapors. In order to avoid the difficulties described, a different method was developed in which the swelling is carried out on finely divided powders of the substance by contact with the vapors of a solvent. This method offers three advantages: No disturbance by crazing, cracking, or leaching occurs; the swelling equilibrium is established rapidly; and precise, gravimetric determination of the amount of swelling is possible.

The samples are ground to micron size, vacuum-dried, and conditioned at 25° C. and 50% relative humidity. Samples of 0.5 gram are weighed into glass jars and placed in a desiccator containing a mixture of 1,2-dichloroethane and cetane (hexadecane) in the molar ratio of 95 to 5. The desiccator is evacuated and kept at constant temperature (25.0° ± 0.3° C.) for 24 hours, and the gain of weight of the samples is determined. The experiment is continued for another 24 hours to ascertain that equilibrium has been established. Condensation of solvent, a potential source of error, was avoided by keeping the temperature constant, by diluting the solvent (dichloroethane) with a nonsolvent (cetane), and by releasing the vacuum slowly.

The results obtained by this method were very satisfactory. The final value was always obtained within 24 hours and the reproducibility of the results was within a few tenths of 1%. The last column of Table II shows swelling values obtained by this method; they are expressed as per cent gain of weight and vary from 60.5% for an inadequately cured resin to 37.6% for a very well cured resin.

Discussion. It would be valuable to convert these swelling values to another value more directly representative of the degree of cross linking, such as the average molecular weight between cross links. However, this has not been possible as yet, partly because the available theoretical treatment applies primarily to slightly cross-linked resins, partly because the conversion requires the knowledge of an interaction constant which is difficult to determine.

Since the swelling values are considered to be a measure for the degree of cross linking (within groups of similar chemical composition), the results were compared with the softening temperatures obtained by the indentation method, a method also believed to furnish a measure for the degree of cross linking. In Figure 7 the upper part illustrates swelling values, the lower part softening temperatures obtained on the same samples. Though a perfect agreement of the two sets of curves does not exist (and cannot be expected), the similarity is unmistakable. This appears to strengthen the opinion that either method gives results that can be considered, in a relative way, to be estimates of the degree of cross linking and can be used, within groups of similar chemical composition, for a comparison and evaluation of cured thermosetting resins.

Table II. Softening Temperatures and Swelling Values of Cured Epon 828

Curing Agent, Parts/100 Parts Epon	Cure Time, Hours	Cure Temp., ° C.	Arbitrary Softening Temp., ° C.[a]	Swelling Value, %
Piperidine[b], 5	24	65	103	60.5
	+ 4[c]	100	131.5	56.5
	+ 1[c]	150	125.5	54.6
	+ 0.5[c]	200	135	55.2
Tris(dimethylaminomethyl) phenol, 4	24	65	125	51.8
	+ 4[c]	100	153	42.5
	+ 1[c]	150	178	40.5
	+ 0.5[c]	200	199	39.3
m-Phenylenediamine[b], 12.5	24	65	113	49.2
	+ 4[c]	100	161	41.6
	+ 1[c]	150	185	39.2
	+ 0.5[c]	200	229	37.6

[a] For indentation of 0.0075 inch within 20 seconds.
[b] Pure compound.
[c] Cures in addition to cures indicated in line or lines above.

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LITERATURE CITED

(1) Am. Soc. Testing Materials, "ASTM Standards," vol. 6, p. 1176, Method D 648-45 T, 1952.
(2) *Ibid.*, p. 736, Method D 1238-52 T, 1952.
(3) Boyer, R. F., and Spencer, R. S., *J. Polymer Sci.* 3, 97 (1948).
(4) Crank, J., *Ibid.*, 11, 151 (1953).
(5) Flory, P. J., "Principles of Polymer Chemistry," chap. XI and XIII, Cornell Univ. Press, Ithaca, N. Y., 1953.
(6) Forziati, F. H., Stone, W. K., Rowen, J. W., and Appel, W. D., *J. Research Natl. Bur. Standards* 45, 109 (1950), Research Paper RP2116.
(7) Harp, W. R., Stone, H., and Otvos, J. W., "Quantitative Absorption Spectrometry of Solids," Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., March, 1954.
(8) Herzberg, G., "Infrared and Raman Spectra," p. 340, Van Nostrand, New York, 1945.
(9) Heuser, E., "Chemistry of Cellulose," p. 240, Wiley, New York, 1944.
(10) Jungnickel, J. L., Peters, E. C., Polgar, A., and Weiss, F. T., "Organic Analysis," vol. I, p. 135, Interscience, New York, 1953.
(11) Kienle, R. H., *Ind. Eng. Chem.* 22, 590 (1930).
(12) King, G., *Nature* 164, 706 (1949).
(13) *Paint, Oil, Chem. Rev.*, p. 35, (June 28, 1945).
(14) Patterson, W. A., ANAL. CHEM. 26, 823 (1954).
(15) *Perkin-Elmer Instrument News* 4, 1 (1953).
(16) Rooney, J. H., and others, "Investigation of German Plastic Plants," Part 2, U. S. Office of Publication Board, Rept. No. 25642; *Mod. Plastics* 24, 254 (1947) (abstract).
(17) Russell, E. W., *Nature* 165, 91 (1950).
(18) Sauer, J. A., and Hsiao, C. C., *India Rubber World* 128, 355 (1953).
(19) Schiedt, V., *Z. Naturforsch.* 76, 270 (1952).
(20) Stimson, M. M., and O'Donnell, M. J., *J. Am. Chem. Soc.* 74, 1805 (1952).

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New Iodometric Determination of Cobalt Based on Formation of Iodopentamminecobalt(III) Nitrate

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The reaction between cobalt and iodine in ammoniacal solutions of ammonium nitrate to form iodopentamminecobalt(III) nitrate can be used for the determination of cobalt(II). The reaction is rapid and oxygen does not need to be excluded. The end point can be determined either potentiometrically or by using the starch indicator. Copper, nickel, etc., do not interfere, nor do aluminum, iron, and chromium in the presence of tartrate.

WHEN iodine is added to ammoniacal solutions of cobalt containing ammonium nitrate, chloride, bromide, or iodide, the formation of the corresponding salt of iodopentamminecobalt(III) occurs (*9*). The preparation of these compounds is direct and rapid, and does not require the exclusion of air. Because the direct ferricyanide titration of cobalt either as the cobalt(II)–ammonia (*1*, *6*) or the cobalt(II)–ethylenediamine (*2*) complex does require the removal of air from the initial solutions, it seemed worth while to investigate the analytical possibilities of the reaction involved in the formation of the iodopentamminecobalt(III) salts. Further studies are needed.

EXPERIMENTAL

It was found that iodopentamminecobalt(III) is stable in the presence of arsenic(III). Accordingly, an excess of iodine was used in each experiment and, after the reaction had occurred, the solutions were titrated with standard arsenic(III). Because the solutions of the iodo complex ion are yellowish green and the iodo complex hydrolyzes to form the red-violet hydroxopentamminecobalt(III), the end point of the iodine-arsenic reaction was determined potentiometrically. It was subsequently found that the starch end point could also be used. Attempts to determine cobalt gravimetrically by the formation of iodopentamminecobalt(III) salts were unsuccessful.

Apparatus. A Beckman Model G pH meter was used as a potentiometer. Bright platinum and calomel electrodes having 24-inch shielded leads were used. The titrations were done in a three-necked 200-ml. round-bottomed flask and the solutions were magnetically stirred.

Reagents. COBALT(II) SULFATE. A desired amount of reagent grade cobalt(II) sulfate was dissolved in 2 liters of distilled water. The solution was standardized by determining the cobalt content in 100-ml. aliquots by the method of Smith (*5*). A precision within 0.025% was observed. Aliquots of the stock solution were diluted as required.

IODINE. The standard technique for preparing iodine solutions from resublimed iodine and reagent grade potassium iodide was followed. However, it was found that in the ammoniacal solutions better results were obtained when twice the usual amount of potassium iodide was present. Thus for the preparation of 0.05*N* iodine solutions, 6.3 grams of iodine and 25 grams of potassium iodide per liter of solution were used. The iodine solutions were standardized potentiometrically against standard arsenic(III) using sodium bicarbonate. The same titer was observed with an ammonia–ammonium ion buffer at a pH of 9. These solutions were restandardized each time they were used.

STANDARD ARSENIC(III). A solution of 0.1000*N* arsenic(III) was prepared from National Bureau of Standards arsenic trioxide according to their directions. Aliquots were diluted to prepare solutions of 0.05000 and 0.01000*N* arsenic(III).

Potentiometric Method Adopted. To 25 ml. of a solution containing from 1 to 250 mg. of cobalt in a round-bottomed, three-necked flask add 25 grams of ammonium nitrate and 25 ml. of an iodine solution of sufficient strength, so as to provide a 10 to 50% excess. After all of the ammonium nitrate has been dissolved with the aid of magnetic stirring, add 5 ml. of concentrated ammonia. Solutions which are acid may be conveniently neutralized by the addition of a 6*N* ammonium carbonate solution until the formation of carbon dioxide stops. The resulting ammoniacal solutions, which will have a pH of about 9, should not be heated nor, unless a very large amount of acid has been neutralized, do they need to be cooled. The precipitation of apple green iodopentamminecobalt(III) nitrate will begin as soon as the ammonia has been added and will be complete within 5 minutes. The excess of iodine is then determined potentiometrically by the addition of a standard arsenic(III) solution. The end-point potential in these titrations was the same as that observed during the standardization of the iodine solutions (+0.27 volt *vs.* SCE).

The results obtained are listed in Table I. When the same procedure was used with a starch end point, less satisfactory results were obtained (Table II). In these experiments 5 ml. of starch solution were added just before the end point was reached and the reaction vessel was a 250-ml. Erlenmeyer flask. When large amounts of cobalt were present, the bulky green precipitate of iodopentamminecobalt(III) nitrate made the end point difficult to observe. By filtering the precipitate onto a medium-pore, sintered-glass Gooch crucible, washing with two small portions of 2*M* ammonium nitrate, and combining the washings with the original filtrate, a sharper end point was obtained.

INTERFERENCES WITH METHOD

Although an exhaustive study of the metals and of the various complexing agents was not made, it is evident that any substance which can oxidize iodide or cobalt (permanganate, persulfate, etc.) or reduce iodine (cyanide) must be absent. Many metals which interfere in their lower oxidation states do not do so in their highest oxidation state. These include arsenate, antimonate, chromate, molybdate, vanadate, and tungstate. Cad-

Table I. Potentiometric Determination of Cobalt

Cobalt Taken, Mg.	pH	Cobalt Found, Mg.	Relative Error, %
64.68	8.8	64.65	−0.05
	9.0	64.68	0.00
	8.8	64.71	0.11
	9.0	64.71	0.11
16.17	8.8	16.16	−0.10
	8.9	16.15	−0.20
	8.9	16.18	0.10
	8.9	16.18	0.10
3.234	9.0	3.243	0.28
	9.0	3.219	−0.46
	9.0	3.215	−0.60
	9.0	3.227	−0.22

Table II. Determination of Cobalt Using Starch Indicator

Cobalt Taken, Mg.	Cobalt Found, Mg.	Relative Error, %
59.00	58.55	−0.75
	58.72	−0.46
	58.85	−0.25[a]
	59.13	0.22[a]
	59.80	−0.32[a]
11.80	11.71	−0.77
	11.82	0.20
	11.76	−0.36
	11.73	−0.60
2.950	2.915	−1.16
	2.932	−0.60
	2.943	−0.24
	2.927	−0.78

[a] Precipitate filtered before titrating.

mium, copper, nickel, and zinc which form ammino complexes or mercury(II) which forms an iodo complex do not interfere in the presence of excess ammonia or iodide, respectively. However, elements which form insoluble hydroxides or iodides in ammoniacal solutions will carry down cobalt and interfere with the proposed method. Metals in this group include aluminum, bismuth, chromium, iron, tin, silver, lead, mercury(I), and manganese.

The results of a number of experiments on the prevention of interference by various metallic ions on the proposed iodometric determination of cobalt are summarized in Table III and are discussed in more detail below.

Effect of Presence of Nickel, Copper, Zinc, and Cadmium. These elements form ammino complexes and, as a result, lower the pH of the solutions. It was found that when the pH of the solution was less than 8.5, the rate of formation of iodopentamminecobalt(III) was slow and the analysis was low. This can be remedied by the addition of sufficient ammonia to raise the pH to 9 (Table IV). Because of the deep blue color of the copper-ammine complex ion, the starch end point cannot be used in the determination of cobalt in the presence of copper.

Effect of Presence of Silver, Lead, and Mercury. Silver, lead, and mercury(I) will precipitate as the iodides in acid solutions. The precipitates do not occlude cobalt. However, they cause a loss of iodide ion and increase the hydrolysis of iodine in the ammoniacal solutions and low results in the cobalt determination will be obtained. This may be remedied by precipitating these elements by the addition of potassium iodide before the addition of the iodine solutions. When mercury(II) is present, the stable mercury-iodide complex is formed and more iodide must also be added.

Effect of Presence of Aluminum, Bismuth, Chromium, Iron, and Tin. In the presence of these elements which form insoluble hydroxides in ammoniacal solutions it was necessary to add a complexing agent in order to prevent the occlusion of cobalt (Table V). Fluoride, oxalate, and pyrophosphate, which do not interfere with the formation of iodopentamminecobalt(III), were unsatisfactory. Ethylenediaminetetraacetic acid forms a very stable complex with cobalt(II) and interferes with the formation of the iodo complex. The oxidation of the cobalt–ethylenediaminetetraacetic acid complex by iodine occurs very slowly. Although citrate does not interfere with the formation of iodopentamminecobalt(III), its reaction with iodine to form tetraiodoacetone and iodoform is catalyzed by the presence of cobalt as well as other metals (*4*). Thus in the presence of chromium, iodoform is quickly formed. Satisfactory results can be obtained by using tartrate, which is added as tartaric acid to the initial solutions. The results of a number of experiments on the determination of cobalt in the presence of chromium are listed in Table V. Similar observations were made with aluminum, bismuth, iron, and tin.

Manganese. In the ammoniacal solutions manganese(II) was oxidized by iodine to a mixture of manganese(III) and manganese(IV) hydroxides. In the presence of tartrate oxidation of manganese(II)–tartrate to manganese(III)–tartrate complex occurred. In the absence of oxygen only partial oxidation occurred. Attempts to prevent completely the oxidation of manganese(II) in the presence of tartrate were unsuccessful. The reaction appeared from the few experiments carried out here to be oxygen catalyzed. In order to determine cobalt directly by the formation of iodopentamminecobalt(III), manganese must be removed as the dioxide in strongly acid solutions.

Table III. Effect of Metallic Ions on Iodometric Determination of Cobalt

Ion	Amount, Mg.	Interference	Reagent Required
As(V), Sb(V), V(V), Cr (VI), Mo(VI), W(VI)	500	–	
Cu, Cd, Ni, Zn	100	–	
	500	+	Addn. ammonia
Hg(I), Hg(II), Pb, Ag	100	–	
	500	+	Addn. iodide
Al, Bi, Cr(III), Fe, Sn	100	+	Tartrate
Mn	25	–	

Table IV. Analysis of Cobalt Solutions Containing Cadmium, Copper, Nickel, Zinc, and Mercury

Cobalt Present, Mg.	Element Present, Mg.		pH	Cobalt Found, Mg.	No. of Expts.
6.40	Copper	1320	6	0.00	2
6.40	Copper	1320	8.4	6.20	2
6.40	Copper	1320	8.8	6.42	2
6.40	Cadmium	200	8.8	6.38	2
6.40	Nickel	224	9.0	6.43	2
6.40	Zinc	224	8.8	6.37	2
6.40	Mercury(II)	100	8.8	0.0	2
6.40	Mercury(II)	100	9.0[a]	6.37	2

[a] 0.500 gram of potassium iodide added.

Table V. Determination of Cobalt in Presence of Chromium

Cobalt Present, Mg.	Chromium Present, Mg.	Complexing Agent	Cobalt Found, Mg.	No. of Expts.
64.0	100		5.2	2
64.0	100		9.1[a]	2
64.0	100		62[b]	2
64.0	100	EDTA	2.4	2
64.0	100	Citrate	74	2
64.0	100	Tartrate	63.8	2

[a] Amount of cobalt after aging of chromium hydroxide for 1 hour.
[b] Cobalt added after precipitation of chromium.

DISCUSSION

The formation of iodopentamminecobalt(III) may be written as follows:

$$2Co^{++} + 10\ NH_3 + I_3^- = 2Co(NH_3)_5I^{++} + I^- \qquad (1)$$

This reaction is reversible (*8*) and when the reduced solutions are allowed to stand, the starch-iodine color slowly forms. This may be the cause of the low results observed when the amount of cobalt present is of the order of 10 mg. or less.

In order to prevent the reduction of iodopentamminecobalt(III) by iodide ion a series of experiments was carried out using iodine dissolved both in alcohol and in concentrated ammonium bromide solutions. Under these conditions iodine will hydrolyze to a greater extent than it will in solutions containing iodide ion and the hypoiodite ion formed may be air oxidized to iodate ion (*3*). Because the latter ion does not react with cobalt(II), these experiments were performed in a hydrogen atmosphere. Although a number of factors such as the order of addition of the reagents and the length of time, the temperature and the pH of the reaction were also varied, only 30 to 95% of the cobalt present could be found.

The oxidation of cobalt(II) by hypoiodous acid ion was also investigated. Iodine monochloride, iodine dissolved in silver nitrate solutions, and iodine shaken with mercuric oxide were used as sources of hypoiodous acid. The reactions were carried out in oxygen-free solutions buffered with ammonia and ammonium salts at pH's of 9 to 9.5. With small amounts of cobalt the same yellow-brown color that occurs during the air oxidation of cobalt-ammonia solutions was observed; in more concentrated solutions both iodopentamminecobalt(III) nitrate and decammine μ-peroxo dicobalt(III) nitrate were formed. Separate experiments with the latter binuclear complex prepared according to the direction of Werner (*7*) showed that it reacts rapidly and irreversibly with solutions of iodine according to the equation:

$$(NH_3)_5CoOOCo(NH_3)_5^{++++} + I_2 = O_2 + 2Co(NH_3)_5I^{++} \qquad (2)$$

The occurrence of these reactions, therefore, would give high rather than low cobalt results.

Table VI. Effect of Oxygen on Determination of Cobalt

(Rate of bubbling, 2 ml. per minute)

Cobalt Present, Mg.	Cobalt Found,[a] Mg.	Time, Minutes	No. of Expts.
11.80	11.78	1	2
	11.75	20	2
	11.55	120	2

[a] Iodine solutions added after oxygen was bubbled through ammoniacal solutions of cobalt.

If, on the other hand, decammine μ-peroxo dicobalt(III) were formed by the air oxidation of cobalt(II) ammines and subsequently was converted, perhaps catalytically, into hydroxopentamminecobalt(III) or hexamminecobalt(III) by some reaction other than that of Equation 2, then low cobalt results would be served. A series of experiments was carried out in order to study the stability of decammine μ-peroxo dicobalt(III) using the following procedure:

Oxygen, which had previously been passed through a solution of ammonium hydroxide, was bubbled through 25 ml. of a solution of cobalt sulfate containing 25 grams of ammonium nitrate and 5 ml. of concentrated ammonia at the rate of 2 ml. per minute. After a predetermined length of time, 25 ml. of a standard iodine solution were added and the excess iodine was determined potentiometrically in the usual way.

The results of these experiments, recorded in Table VI, indicate that decammine μ-peroxo dicobalt(III) ion is very stable in the dilute cobalt and ammonia solutions present in the proposed analytical procedure.

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LITERATURE CITED

(1) Dickens, P., and Maassen, G., *Arch. Eisenhüttenw.* **9**, 487 (1935).
(2) Diehl, H., and Butler, J. P., ANAL. CHEM. **27**, 777 (1955).
(3) McAlpine, R. K., *J. Chem. Educ.* **26**, 362 (1949).
(4) Qureshi, M., and Veeraiah, K., *Current Sci. (India)* **15**, 132 (1946).
(5) Smith, J. D. M., *Chem. Inds.* **44**, 5391 (1925).
(6) Tomicek, O., and Freiberger, F., *J. Am. Chem. Soc.* **57**, 801 (1935).
(7) Werner, A., *Ann.* **375**, 1 (1910).
(8) Yalman, R. G., *J. Am. Chem. Soc.* **75**, 1842 (1953).
(9) Yalman, R. G., *Ibid.*, **77**, 3219 (1955).

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Interaction of Platinum Group Elements with 1,2,3-Benzotriazole

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This paper is part of a general study of the interactions of the platinum metals with 1,2,3-benzotriazole. Methods for the gravimetric and for the amperometric determination of palladium(II) chloride, in acetic acid–sodium acetate buffer, have been developed using 1,2,3-benzotriazole as a precipitant. From 3 to 60 mg. of palladium were determined gravimetrically with an average error of less than 0.1 mg. The amperometric method has been applied to 0.2 to 6 mg. of palladium. When the concentration of palladium is greater than 0.2 m*M*, the average relative analytical error is within ±0.3%. The methods are recommended primarily for the accurate determination of chloride solutions of palladium containing only traces of other platinum elements.

WHILE studying the interaction of palladium (II) and 1,2,3-benzotriazole (*15*) it was observed that palladium was precipitated quantitatively in this reaction. 1,2,3-Benzotriazole has been studied as a precipitant for silver by Remington and Moyer (*11*), Tarasevich (*12*), and Cheng (*2*). Curtis (*4*) has examined this reagent as a precipitant for copper. These studies indicate that iron(II), nickel(II), cobalt(II), zinc, and cadmium are also precipitated by this reagent.

When an excess of 1,2,3-benzotriazole is added to an acetic acid–sodium acetate buffer solution containing palladium(II) chloride in the absence or presence of sodium dihydrogen (ethylenedinitrilo)tetraacetic acid, a white colored coordination compound is formed, which, when dried, corresponds to the formula $Pd(C_6H_4NHN_2)_2Cl_2$ (*15*). In excess palladium, 1,2,3-benzotriazole forms a reddish-brown colored coordination compound; its formula is $Pd(C_6H_4NHN_2)Cl_2$ (*15*).

GRAVIMETRIC DETERMINATION OF PALLADIUM

The gravimetric determination of palladium has been the subject of several investigations during the past few decades. Palladium has been determined gravimetrically with salicylaldoxime (*7*), with β-furaldoxime (*5*), with 1,2-cyclohexanedione (*14*), with dimethylglyoxime (*16*), and with phenylthiourea, thiophenol, and thiobarbituric acid (*3*). Of these gravimetric methods, the dimethylglyoxime procedure has been used very widely.

It was the purpose of this investigation to ascertain the possibility of obtaining reproducible and sufficiently accurate stoichiometric results in the direct gravimetric determination of palladium(II) with 1,2,3-benzotriazole in acetic acid–sodium acetate buffer, and to determine the extent of interferences from certain diverse ions on the determination of palladium.

EXPERIMENTAL

Reagents and Solutions. A 5-gram sample of palladium(II) chloride, obtained from Coleman and Bell Co., was dissolved in 10 ml. of concentrated hydrochloric acid and diluted to 1 liter with distilled water. This solution was standardized gravimetrically, using modifications of the Gilchrist-Wichers procedure (*6*).

Reagent grade rhodium(III), iridium(IV), platinum(IV), ruthenium(III), iron(III), chromium(III), aluminum(III), zinc, magnesium, nickel(II), and cobalt(II) chlorides were used to prepare solutions of these elements. Osmium(VIII) solution was prepared from osmium tetroxide, using the procedure of Ayres and Wells (*1*). Platinum(II) solution was prepared from potassium tetrachloroplatinate(II). Gold(III) solution was prepared from chloroauric acid trihydrate.

1,2,3-Benzotriazole, Eastman Kodak Chemical No. 2759, was recrystallized twice from chloroform and dried at room temperature. A weighed amount was dissolved in 125 ml. of glacial acetic acid and diluted to 250 ml. with distilled water. The concentration of this solution was checked by modifying the method of Cheng (*2*); the results obtained by this method agree closely with the calculated concentration of 1,2,3-benzotriazole.

(Ethylenedinitrilo)tetraacetic acid (Versenate) solution was prepared by dissolving 40 grams of the disodium salt of (ethylenedinitrilo)tetraacetic acid, analytical reagent grade from Versenes, Inc., in 1 liter of distilled water.

A buffer solution was prepared, which was 2*M* in acetic acid and in sodium acetate.

Qualitative Study of Interaction of 1,2,3-Benzotriazole with Platinum Metals. Preliminary qualitative tests were carried out using individual solutions of the platinum elements employing 1,2,3-benzotriazole as a possible reagent for the qualitative or quantitative determination of these metallic ions. These exploratory tests were undertaken to determine the nature of these reactions and to ascertain whether or not the properties of the systems might offer possibilities for further investigation.

The interactions of the platinum elements, after the solutions had been diluted so that concentration of the ions was of the order of $1 \times 10^{-3}M$, with 1,2,3-benzotriazole was studied in the absence and in the presence of disodium dihydrogen (ethylenedinitrilo)tetraacetic acid. At room temperature (about 25° C.) and in the absence of disodium dihydrogen (ethylenedinitrilo)-tetraacetic acid, 1,2,3-benzotriazole reacts with palladium(II) to give a white precipitate which remains suspended in solution, for at least several hours. All test solutions in which Versenate was employed contained about a five-fold molar excess of this reagent. In the presence of disodium dihydrogen (ethylenedinitrilo)tetraacetic acid, palladium begins to precipitate only after the addition of nearly equal molar amounts of 1,2,3-benzotriazole. However, in the absence of disodium dihydrogen (ethylenedinitrilo)tetraacetic acid, palladium begins to precipitate immediately after the initial addition of a very small quantity of reagent. The fact that palladium(II)-Versenate complex is more stable than the palladium(II)-chloride complex has been reported (*10*). After being heated in a water bath at 85° C. for 10 minutes, the palladium(II)-chloride–1,2,3-benzotriazole precipitate was slightly coagulated; however, the precipitate coagulated when disodium dihydrogen (ethylenedinitrilo)tetraacetic acid was present.

In the absence of disodium dihydrogen (ethylenedinitrilo)-tetraacetic acid at room temperature, the ruthenium–1,2,3-benzotriazole system gives an intense rose-red color, and the osmium–1,2,3-benzotriazole system gives a pale yellow color. These two elements gave no visible reaction with this reagent in the presence of disodium dihydrogen (ethylenedinitrilo)tetra-

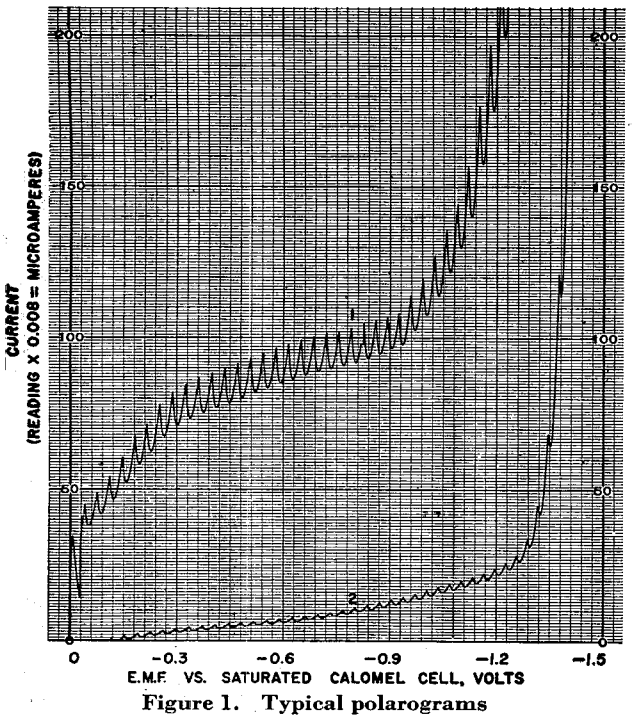

Figure 1. Typical polarograms

1. $0.0865 \times 10^{-2}M$ palladium(II) in $0.4M$ acetic acid–sodium acetate buffer
2. $0.257 \times 10^{-2}M$ 1,2,3-benzotriazole in $0.4M$ acetic acid–sodium acetate buffer

Table I. Gravimetric Determination of Palladium

Pd Taken, Mg.	Wt. of Ppt., Mg.	Pd Found, Mg.	Difference, Mg.
2.97	11.5	2.95	−0.02
2.97	11.6	2.98	+0.01
2.97	11.4	2.92	−0.05
8.91	34.8	8.93	+0.02
8.91	34.5	8.85	−0.06
8.91	34.6	8.89	−0.02
14.85	58.2	14.93	+0.08
14.85	57.8	14.83	−0.02
14.85	57.9	14.86	+0.01
29.70	115.5	29.64	−0.06
29.70	115.7	29.69	−0.01
29.70	115.6	29.66	−0.04
44.55	174.1	44.67	+0.12
44.55	173.0	44.39	−0.16
44.55	173.8	44.60	+0.05
59.40	232.1	59.56	+0.16

Table II. Gravimetric Determination of Palladium in Presence of Diverse Ions

Pd Taken, Mg.	Ion Added, Mg.	Pd Found, Mg.	Difference, Mg.
14.85	9.3 Pt(IV)	14.81	−0.04
14.85	10.1 Ir(IV)	14.78	−0.07
14.85	7.8 Rh(III)	14.88	+0.03
14.85	8.9 Os(VIII)	14.93	+0.08
14.85	9.4 Ru(III)	14.99	+0.14
14.85	5.6 Fe(III)	14.86	+0.01
14.85	2.8 Al(III)	14.83	−0.02
14.85	6.5 Zn(II)	14.88	+0.03
14.85	2.4 Mg(II)	14.86	+0.01
14.85	5.9 Ni(II)	14.65	−0.20
14.85	6.0 Co(II)	14.83	−0.02
14.85	12.4 NO_3^-	14.86	+0.01
14.85	19.2 SO_4^{--}	14.83	−0.02

acetic acid; this probably indicates that the Versenate complexes of these ions, if they exist, are rather stable. On heating the solutions which contained no disodium dihydrogen (ethylenedinitrilo)tetraacetic acid, the ruthenium–1,2,3-benzotriazole system changed from rose-red to bluish green and the osmium–1,2,3-benzotriazole system changed from pale yellow to orange. After digesting these solutions in the usual way with disodium dihydrogen (ethylenedinitrilo)tetraacetic and excess 1,2,3-benzotriazole, osmium gave no visible reaction and ruthenium gave a brown precipitate which formed slowly on standing. Of the other platinum metals no visible reaction was obtained with or without disodium dihydrogen (ethylenedinitrilo)tetraacetic acid at room temperature. Rhodium(III) and platinum(II) during digestion reacted very slowly, in the absence of disodium dihydrogen (ethylenedinitrilo)tetraacetic acid, to form precipitates which remained suspended in solution; in the presence of disodium dihydrogen (ethylenedinitrilo)tetraacetic acid, rhodium gave no visible reaction and platinum(II) gave a small amount of turbidity which formed on standing.

Further tests showed that a high concentration of acetate ion was very effective in coagulating the palladium(II) chloride–1,2,3-benzotriazole precipitate. It was found that this precipitate coagulates immediately when an acetic acid solution of this reagent is added to an acetic acid–sodium acetate buffer solution containing palladium, in either the presence or absence of Versenate.

Properties of Palladium(II) Chloride–1,2,3-Benzotriazole Precipitates. When palladium is precipitated by 1,2,3-benzotriazole in the presence of excess reagent, the ratio of reagent to palladium is 2 to 1; the precipitate exhibits a white color, both in solution and after drying. In excess palladium, the reagent to palladium ratio is 1 to 1 (*15*). Careful observation shows that in solution when palladium combines with 1,2,3-benzotriazole in the ratio of 1 to 1, the precipitate appears beige; however, after drying the precipitate appears reddish brown.

Temperatures from 110° to 150° C. were safe for drying the precipitates. Constant weight was obtained for the precipitates after they had been heated at 110° C. for 30 minutes. The pre-

cipitates did not show sensitivity to diffuse light. The precipitates were practically insoluble in most concentrated inorganic acids and in most organic solvents.

The precipitates are readily coagulated by acetic acid–sodium acetate buffer. However, when interfering ions that are complexed by disodium dihydrogen (ethylenedinitrilo)tetraacetic acid in acid media are present, it is desirable to carry out the precipitation in disodium dihydrogen (ethylenedinitrilo)tetraacetic acid solution.

Recommended Procedure. To a solution containing from 2 to 50 mg. of palladium are added 10 ml. of 2*M* acetic acid–sodium acetate buffer, and 5 to 10 ml. of 4% disodium dihydrogen (ethylenedinitrilo)tetraacetic acid, depending upon the amounts of interfering metals present. Then a slight excess of a 2.5% 1,2,3-benzotriazole (dissolved in 50% acetic acid) solution is added, and the solution is digested between 60° and 90° C. for 10 minutes to coagulate the precipitate. The cooled, digested precipitate is then filtered using suction through a weighed, medium porosity, sintered-glass crucible or a weighed Gooch crucible which contains an asbestos mat. The precipitate is washed several times with dilute hydrochloric acid (1 to 100) and finally several times with distilled water. The precipitate is dried at 110° C. for 1 hour to constant weight. The theoretical factor for palladium, corresponding to the formula $Pd(C_6H_4NHN_2)_2Cl_2$, is 0.2566, the value that is used to calculate the palladium content of the precipitate. Experimental data were in good agreement with the theoretical factor 0.2566.

The data obtained from several determinations of palladium with 1,2,3-benzotriazole, in the absence of and in the presence of interfering ions, are shown in Tables I and II.

DISCUSSION

A method for the gravimetric determination of palladium is presented, which involves the precipitation of palladium as palladium(II) chloride–1,2,3-benzotriazole, $Pd(C_6H_4NHN_2)_2Cl_2$. The method is primarily recommended for the accurate determination of chloride solutions of palladium containing only traces of other platinum elements. However, platinum(II) and gold(III), in moderate amounts, interfere with the determination of palladium. Moderate amounts of sulfate and nitrate ions do not interfere with the determination of palladium(II) chloride.

This method is believed to offer certain advantages over the generally accepted dimethylglyoxime procedure. The precipitate has a larger formula weight and hence a smaller palladium content. The palladium(II) chloride–1,2,3-benzotriazole procedure, as compared to the dimethylglyoxime procedure, saves time and the precipitate is easier to handle.

AMPEROMETRIC DETERMINATION OF PALLADIUM

Toropova and Yakovleva (*13*) have performed amperometric titrations of palladium with salicylaldoxime and with mercaptobenzenethiazole. Kolthoff and Langer (*8*), while investigating the amperometric titration of cobalt with 1-nitro-2-naphthol from a few experiments, observed that palladium could also be titrated with the same reagent. The present investigation is concerned with the amperometric titration of palladium(II) chloride with 1,2,3-benzotriazole.

EXPERIMENTAL

Apparatus. A Sargent Model XII photographic recording polarograph was used to carry out all amperometric titrations herein reported. Polarograms of palladium and of 1,2,3-benzotriazole, in acetic acid–sodium acetate buffer, were recorded employing a Sargent Model XXI visible recording polarograph. The titration cell contained a saturated calomel reference electrode in one compartment, as described by Kolthoff and Lingane (*9*). All current measurements were taken at 25° ± 0.1° C., and potentials are expressed *vs.* the saturated calomel electrode (S.C.E.).

The characteristics of the capillary used were: $m = 0.890$ mg. sec.$^{-1}$; $t = 8.15$ sec. (open circuit in distilled water); $m^{2/3}$ sec.$^{1/6} = 1.313\ m^{2/3}$ sec.$^{-1/2}$; and $h = 40.4$ cm.

Reagents and Solutions. Preparation and standardization of the palladium(II) chloride solution and of the 1,2,3-benzotriazole solution were described under Gravimetric Determination of Palladium. However, solutions of the reagent and of the palladium used in the amperometric titrations were generally less concentrated than those employed for the gravimetric determination of palladium. The nitrogen, as obtained from the Houston Oxygen Co., was of 99.5% or greater purity. This gas was further purified for polarographic use by passing it through a copper-filled furnace tube, which was maintained at an average temperature of 450° C. All other materials used in the preparation of solutions were reagent grade chemicals.

Amperometric Titration of Palladium(II) Chloride with 1,2,3-Benzotriazole. In Figure 1 are recorded typical polarograms of palladium and of 1,2,3-benzotriazole in acetic acid–sodium acetate buffer. These current-voltage curves indicate that the optimum voltage range for carrying out amperometric studies is from −0.3 to −0.9 volt. After a preliminary study of the palladium(II) chloride–1,2,3-benzotriazole system, the following procedure was adapted for the amperometric determination of palladium.

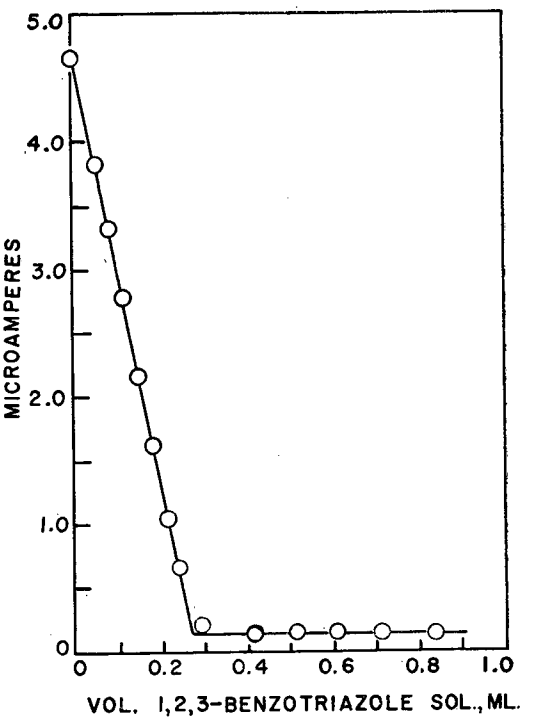

Figure 2. Titration of 20 ml. of 0.0865 × $10^{-2}M$ palladium(II) with 0.0642*M* 1,2,3-benzotriazole

An appropriate aliquot of the stock standard solution of palladium, to give the final concentration desired, was added to a 25-ml. volumetric flask, then 5 ml. of 2*M* acetic acid–sodium acetate buffer and 1 ml. of 0.2% gelatin solution were added; the solution was then diluted to volume with distilled water. A 20-ml. aliquot of this solution was transferred to an H-type cell, and oxygen-free nitrogen, which had been conditioned by passage through a blank solution that contained all of the reagents in the usual concentration except palladium or the titrant, was passed through for 15 minutes. After the passage of nitrogen had been completed, the tip of a microburet containing the 1,2,3-benzotriazole solution was pushed through a hole in the stopper of the titration cell. All current measurements were taken at −0.5 volt and were corrected for dilution effects. After the expiration of a few minutes, the current became constant and the mean of the galvanometer deflections was recorded. A small quantity of the titrant was run into the cell; the solution was stirred with nitrogen for 1 minute, and the current was read 2 minutes thereafter. After each addition of titrant, about 1 minute was required for the current to become constant. This latter procedure was continued throughout the titration. The extrapolation method was employed in ascertaining the volume of titrant used in each titration.

Figure 2 is a typical titration curve of palladium with 1,2,3-

benzotriazole. The results for the amperometric titration of different concentrations of palladium are shown in Table III; each line in the table is the average of two closely agreeing results on samples of the concentration indicated. These data indicate that one palladium ion combines with one molecule of reagent. In Figure 3, the data are recorded for the amperometric titration of 1,2,3-benzotriazole with palladium. When the 1,2,3-benzotriazole is titrated with palladium the ratio of reagent to palladium is 2 to 1.

Effect of Diverse Ions. For application of the method in the analysis of palladium in common compounds of this element, the possible interference from a number of positive and negative ions should be considered. The metallic ions selected for this study were platinum(IV), platinum(II), rhodium(III), iridium(IV), ruthenium(III), osmium(VIII), iron(III), chromium(III), gold(III), aluminum(III), cobalt(II), calcium(II), nickel(II), and magnesium(II). Results of preliminary experiments showed that moderate changes in chloride ion concentration had no measurable effect on the amperometric titration of palladium; therefore, the only negative ions selected for this study were nitrate and sulfate. The interference effect of these ions on the amperometric titration of palladium(II) was studied by adding selected molar concentrations of the diverse ions, individually, to 1 m*M* solutions of palladium (in the final concentration); these solutions were then made up in the usual way. Palladium could not be determined accurately in the presence of osmium(VIII), ruthenium(III), or nickel(II), because the diffusion current was not steady or reproducible when these ions were present in moderate concentrations. When iron(III) or gold(III) were present, the amount of 1,2,3-benzotriazole required for the titration was too large as indicated by the data in Table IV. The presence of the remaining diverse ions produced no measurable effect on the end point for the determination of palladium. Palladium was determined accurately in the presence of trace amounts of cobalt or iridium, but large amounts of these two ions interfered with the titration.

DISCUSSION

An amperometric method for the accurate determination of small concentrations of palladium(II) chloride has been described. The determination is rapid and involves only a few operations. Of the several diverse ions studied, only osmium(VIII), ruthenium(III), iron(III), gold(III), and nickel(II) interfered seriously with the determination of palladium; however, these elements are separated easily from palladium according to the standard procedure (*6*). The data obtained from the amperometric investigation of the palladium(II) chloride–1,2,3-benzotriazole interaction were in agreement with the results obtained from the gravimetric study of the same interaction.

Table III. Amperometric Titration of Palladium(II) in Acetic Acid–Sodium Acetate Buffer at −0.5 Volt *vs.* S.C.E.

Pd Taken, Millimoles × 10^2	1,2,3-Benzotriazole, Millimoles × 10^2	Mole Ratio Pd/Titrant
0.173	0.176	1:1.02
0.346	0.345	1:1.00
1.081	1.084	1:1.00
1.730	1.731	1:1.00
2.594	2.594	1:1.00
3.459	3.494	1:1.01
5.189	5.180	1:1.00

Table IV. Effect of Some Ions on Amperometric Titration of Palladium(II)

Pd Taken, Mg.	Ion Added, Mg.	Pd Found, Mg.	Difference, Mg.
1.846	1.6 Pt(IV)	1.848	+0.002
1.846	1.9 Pt(II)	1.846	0.000
1.846	2.0 Rh(III)	1.844	−0.002
1.846	0.4 Ir(IV)	1.845	−0.001
1.846	0.9 Fe(III)	1.902	+0.056
1.846	1.1 Cr(III)	1.846	0.000
1.846	1.6 Au(III)	1.879	+0.039
1.846	4.7 Co(II)	1.850	+0.004
1.846	0.7 Al(III)	1.845	−0.001
1.846	3.2 Ca(II)	1.847	+0.001
1.846	2.0 Mg(II)	1.845	−0.001
1.846	7.5 SO_4^{--}	1.846	0.000
1.846	5.0 NO_3^-	1.846	0.000

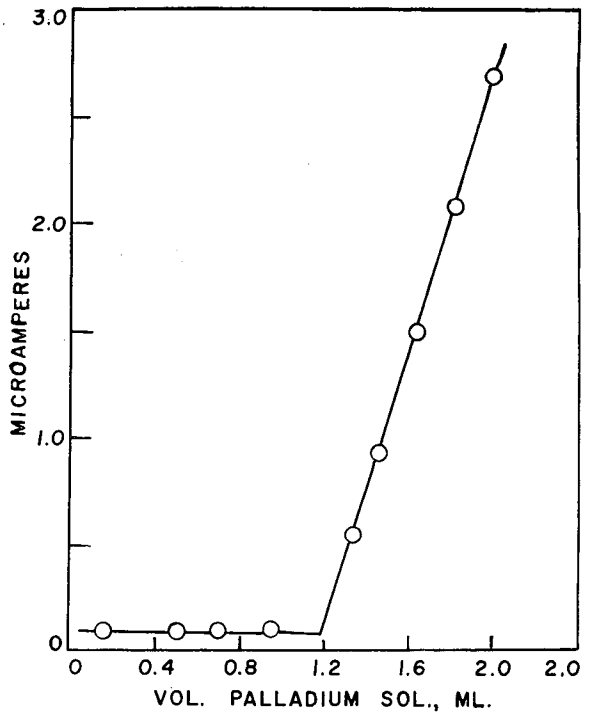

Figure 3. Titration of 20 ml. of 0.257 × 10^{-2}*M* 1,2,3-benzotriazole with 0.0216*M* palladium(II)

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Ayres, G. H., and Wells, W. N., ANAL. CHEM. **22**, 317 (1950).
(2) Cheng, K. L., *Ibid.*, **26**, 1038 (1954).
(3) Currah, J. E., McBride, W. A. E., Cruikshank, A. J., and Beamish, F. E., IND. ENG. CHEM., ANAL. ED. **18**, 120 (1946).
(4) Curtis, J. A., *Ibid.* **13**, 349–51 (1941).
(5) Hayes, J. R., and Chandler, G. C., *Ibid.*, **14**, 491 (1942).
(6) Hillebrand, W. F., Lundell, G. E. F., Bright, H. A., and Hoffman, J. I., "Applied Inorganic Analysis," 2nd ed., pp. 338–83, Wiley, New York, 1953.
(7) Holzer, H., *Z. anal. Chem.* **95**, 392 (1933).
(8) Kolthoff, I. M., and Langer, A., *J. Am. Chem. Soc.* **62**, 3172 (1940).
(9) Kolthoff, I. M., and Lingane, J. J., "Polarography," 2nd ed., vol. 2, p. 903, Interscience, New York, 1952.
(10) MacNevin, W. M., and Kriege, O. H., ANAL. CHEM. **26**, 1768 (1954).
(11) Remington, W. J., and Moyer, H. V., Columbus, Ohio, Ohio State Univ. Press, *Dissertation Abstr.* **24**, (1937).
(12) Tarasevich, N. I., *Vestnik Moskov. Univ.* **3**, No. 10, 161–8 (1948).
(13) Toropova, V. F., and Yakovleva, G. S., *Zhur. Anal. Khim.* **1**, 290 (1946).
(14) Voter, R. C., Banks, C. V., and Diehl, Harvey, ANAL. CHEM. **20**, 652 (1948).
(15) Wilson, R. F., and Wilson, L. E., *J. Am. Chem. Soc.*, in press.
(16) Wunder, H., and Thuring, V., *Chem. Ztg.* **2**, 550 (1912).

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Polarographic Determination of Ferrous and Ferric Iron in Refractory Minerals

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A polarographic method for the simultaneous determination of ferrous and ferric iron in refractory minerals is described. The minerals are initially decomposed in a flux of sodium metafluoroborate. The flux is dissolved in a supporting electrolyte solution containing citrate ion, which complexes both the ferrous and ferric irons. The composite wave of oxidation and reduction of the complex ions gives an anodic current proportional to the concentration of ferrous iron and a cathodic current proportional to the concentration of ferric iron. The procedure can be applied to samples containing both organic materials and manganous compounds.

THERE are several serious limitations (*2*) in the general extensions to rock analysis of the permanganate titration of ferrous iron. First the oxidizability of manganous ion by permanganate in the presence of even limited amounts of hydrofluoric acid tends to give high results in minerals containing manganese; and second any organic matter present tends to cause high results. Further, the simultaneous determination of both ferrous and ferric iron requires a double titration with the attendant complications in the reduction of the ferric iron. A promising rapid polarographic method in which both ferrous and ferric iron are ascertained simultaneously seemed possible from the works of Stackelberg and Freyhold (*7*), Lingane (*4, 5*), and Kolthoff and Lingane (*3*). The composite wave of oxidation and reduction of the complex ions formed between ferric and ferrous ions and oxalate ion give an anodic current proportional to the concentration of the ferrous iron and a cathodic current proportional to the concentration of the ferric iron. Lingane (*5*) indicated that citrate ion might be preferable as a complexing agent for the purpose of determining ferrous and ferric iron.

The purpose of this investigation was to develop a general polarographic method for the assay of the absolute ferric and ferrous iron contents of refractory minerals, as well as the optimal conditions for the dissolution of the mineral sample with minimal oxidation of the ferrous iron.

APPARATUS AND REAGENTS

All polarographic measurements were made on the Sargent Model XXI polarograph. Standard electrolysis cells were used with the dipping-type saturated calomel or silver–silver chloride cells as the reference anode. All measurements were made at 25° ± 0.05° C. The capillary characteristics with a mercury height of 64 cm. in the standard complexing solution on the horizontal portion of the current-voltage curve were: $m = 8.47$ mg. sec.$^{-1}$, $t = 3.5$ seconds, and $m^{2/3}t^{1/6} = 5.12$ mg.$^{2/3}$ sec.$^{-1/2}$

All reagents used were of the highest reagent grade. The distilled water was the equivalent of that produced by a triple distilling process. The supporting electrolyte solution included the complexing agent and had the following composition: sodium citrate, 0.25*M*; citric acid, 0.25*M*; and potassium nitrate, 0.50*M*. The pH of this solution is 4.00 and should be maintained at the same value for all solutions inasmuch as the half-wave potentials are determined by the pH (*5*). The potassium nitrate is added as the carrying electrolyte. Potassium nitrate was chosen in preference to potassium chloride, because there is a maximum in the ferric wave which was difficult to suppress when potassium chloride was used. Gelatin can be used as a maximum suppressor with potassium chloride. However, to reduce the number of reagents used in the analytical procedure, preference was given to the use of potassium nitrate. The flux used for the breakdown of the minerals was sodium metafluoroborate (*6*). This flux has advantage over sodium carbonate in that it gives no effervescence even at high temperatures in producing rapid and complete dissolution of minerals (*1*).

RECOMMENDED PROCEDURE

A weighed sample of 0.1 to 0.2 gram of powdered mineral and about six times its weight of sodium metafluoroborate are placed in a platinum crucible, the weight of which was previously ascertained. The crucible is covered with an inverted clay crucible through which nitrogen gas enters at the top. After a 5-minute nitrogen flush of the apparatus, the flux mixture is gradually brought to a fusion temperature of 1000° to 1050° C. with a Meker burner under an atmosphere of nitrogen. The fused condition is maintained for approximately 5 minutes, or until a clear homogeneous melt is obtained. The crucible is allowed to cool under a stream of nitrogen, weighed again, and the melt removed. The melt is ground under acetone and dried under a stream of nitrogen. An aliquot of 0.3 to 0.4 gram of the powder is weighed and dissolved in 50 ml. of oxygen-free complexing solution under a stream of nitrogen. An aliquot of this amount gives adequate diffusion currents for minerals containing up to 20% total iron. After the solution attains a temperature of 25.00° C., a polarogram is obtained from +0.15 to −0.35 volt (Figure 1). The zero current value is ascertained by the pen position with the dropping mercury electrode polarity switch in the "off" position.

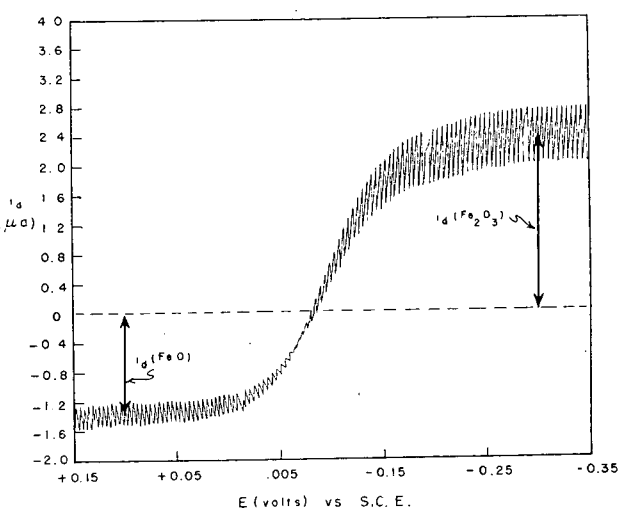

Figure 1. Typical polarogram

Solution contained both divalent and trivalent iron in the supporting electrolyte solution, which contained 0.25*M* citric acid, 0.25*M* sodium citrate, and 0.50*M* potassium nitrate.

To determine the respective polarographic constants, k_{FeO} and $k_{Fe_2O_3}$, for the equation of quantitative polarographic analysis, $i_d = kC$, where i_d is the diffusion current and C the concentration in question, aliquots containing the equivalent of 0 to 8.0 mg. of ferrous and ferric oxides are added to 50-ml. portions of the complexing solution. Reagent grade ferric ammonium sulfate, $Fe_2(SO_4)_3.(NH_4)_2SO_4.24H_2O$, and reagent

grade ferrous ammonium sulfate, $FeSO_4.(NH_4)_2SO_4.6H_2O$, were used as standards. Weighed quantities of these salts were introduced directly into the complexing solutions.

This procedure bypasses the preparation of standard ferrous and ferric solutions; the concentration of the ferrous standard may change with time. It further eliminates the pipetting of standard solutions in which oxidation of ferrous iron may occur.

Table I. Calibration Data for Ferrous and Ferric Oxides[a]

Run	FeO Concn., Mg./50 Ml.	$-id$, μa.	$-id$[b], μa.	k_{FeO}	Fe_2O_3 Concn., Mg./50 Ml.	$+id$, μa.	$+id$[b], μa.	$k_{Fe_2O_3}$
1	0.0	0.20	0.00	...	0.0	0.06	0.00	...
2	1.25	0.96	0.76	0.610	1.13	0.64	0.58	0.515
3	1.67	1.24	1.04	0.624	1.77	0.98	0.92	0.519
4	2.05	1.53	1.33	0.648	1.97	1.06	1.00	0.507
5	2.71	1.84	1.64	0.605	3.51	1.90	1.84	0.524
6	3.54	2.46	2.26	0.639	3.71	1.93	1.87	0.520
7	3.94	2.72	2.52	0.640	3.74	2.02	1.96	0.524
8	4.82	3.26	3.06	0.635	4.21	2.30	2.24	0.533
9	6.10	3.92	3.72	0.610	6.71	3.60	3.54	0.528

[a] In 0.5M potassium nitrate, 0.25M sodium citrate, and 0.25M citric acid. Dropping mercury electrode *vs.* S.C.E. where $M^{2/3}t^{1/6} = 5.12$ mg.$^{2/3}$ sec.$^{-1/2}$. Ferrous and ferric values both obtained in single runs listed above.
[b] Corrected for blank values.

The slight increase of volume due to the introduction of the solid salts into the solution is insignificant. The results of one set of these calibrations are given in Table I. The values of the k's are expressed in microamperes per milligram instead of microamperes per millimole for convenience in the rapid conversion of results to the weight units used in the literature of geology. The standard deviations of k_{FeO} and $k_{Fe_2O_3}$ are 2.6 and 1.6%, respectively.

There apparently is no effect of the ferric iron produced by the oxidation of ferrous iron upon the measured ferric iron value of the sample. This is shown in Table I where each sample contained both ferrous and ferric iron. There is no trend in the values of $k_{Fe_2O_3}$ with increasing ferrous concentration.

Table II. Comparison of Polarographic and Volumetric Determinations of Ferrous Oxide Content in Weight Per Cent

Description of Sample	Polarographic, %	Volumetric, %
Glauconite	12.4	11.5
	12.5	11.1
	12.2	12.5
	11.2	11.6
	11.2	11.6
Olivine	5.01	6.29
	6.55	6.52
Saponite	4.46	4.51
Hornblende	19.88	19.89
Marcasite bearing rocks	19.15	...
	18.93	...

RESULTS

The polarographic determinations of ferrous iron were compared with the results of a permanganate titration of splits from identical samples (Table II). In the volumetric procedure the melt was dissolved in a solution containing 20 ml. of 12N sulfuric acid and 20 ml. of saturated boric acid solution under an atmosphere of nitrogen. The mixture was warmed slightly to effect solution. Upon cooling, 5 ml. of 86% phosphoric acid were added, and the titration was carried out. The results of these titrations are given in Table II and show reasonable agreement with the results of the polarographic method. The titration was not carried to completion for the marcasite sample inasmuch as the hydrogen sulfide formed during acidification reduced permanganate. Replicate analyses were made on the glauconite, olivine, and marcasite samples.

Table III. Determination of Ferrous and Ferric Oxides with Presence of Manganous Ion

$MnCl_2$ Added, Mg.	FeO Present, mg.	FeO Found, mg.	Fe_2O_3 Present, mg.	Fe_2O_3 Found mg.
0	4.82	4.85	2.47	2.51
11.0	4.78	4.76	0.0	0.0
14.4	4.87	4.85	4.09	4.10
22.4	4.93	5.03	2.45	2.49
44.3	4.86	4.85	2.44	2.49

It was found that manganous ion does not interfere with the polarographic determination of either the ferrous or ferric iron inasmuch as the oxidation potential of the manganous ion is far removed from that of the ferrous-ferric waves. Recovery determinations of known quantities of ferrous and ferric oxides with varying quantities of manganous chloride are given in Table III.

The fusion process can be carried out with minimum oxidation of ferrous iron if done rapidly and under a nitrogen atmosphere. However, lengthy grinding periods, even under acetone, result in a reduction of the ferrous concentration (Table IV). To reduce any photochemical reduction of the citrate solutions, it is recommended to carry out the polarographic determinations in low actinic (colored) glass vessels or in the absence of strong light.

Table IV. Oxidation of Ferrous Iron During Grinding Process

Description of Sample	Per Cent FeO	Per Cent Fe_2O_3
Glauconite chips (directly from crucible)	12.60	6.6
Glauconite chips (broken up by light pounding in a mortar, without acetone)	12.40	7.1
Glauconite powder (ground in acetone, 20 mesh)	11.93	7.6
Glauconite powder (ground in acetone, 100 mesh)	11.22	7.7
	11.19	7.6

CONCLUSION

The use of the polarographic composite oxidation and reduction waves of ferric and ferrous ions provides a convenient method of assay in refractory materials even in the presence of manganous ion and organic matter. The use of the sodium metafluoroborate flux, with the precautions of limited light exposure, fusion time, and grinding, results in minimum oxidation of ferrous iron.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Groves, A. W., "Silicate Analysis," 2nd ed., p. 184, George Allen and Unwin, Ltd., London, 1951.
(2) Hillebrand, W. F., and Lundell, G. E. F., "Applied Inorganic Analysis," 2nd ed., p. 912, Wiley, New York, 1953.
(3) Kolthoff, I. M., and Lingane, J. J., "Polarography," 2nd ed., p. 219, Interscience, New York, 1952.
(4) Lingane, J. J., *Chem. Revs.* **29**, 17 (1941).
(5) Lingane, J. J., *J. Am. Chem. Soc.* **68**, 2448 (1946).
(6) Rowledge, H. P., *J. Roy. Soc. W. Australia* **20**, 165 (1934).
(7) Stackelberg, M. von, and Freyhold, H. von, *Z. Elektrochem.* **46**, 120 (1940).

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Determination of Vapor-Liquid Ratio of Motor Gasoline

Sunbury Apparatus

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The Sunbury apparatus for the determination of the vapor-liquid ratio of motor gasoline is described, with full details of its method of use. Experimental data indicate that the repeatability of the method is ±0.5° F. at vapor-liquid ratios of between 10 and 40 and that the corresponding value for reproducibility is ±1.0° F. Evidence of the suitability of the apparatus is provided by the satisfactory agreement between experimental and calculated theoretical vapor-liquid ratios for a blend of pure hydrocarbons.

THE term "vapor locking" is currently used to denote an adverse change in the performance of the fuel system of an engine resulting from the evolution of vapor from the fuel. In the engine, vapor lock depends on the amount of vapor formed and on the ability of the fuel system to handle the vapor.

Practical experience has shown that the distillation and vapor pressure characteristics of the fuel are not suitable criteria for vapor-lock control. Apart from expensive and time-consuming road tests, a proper estimate of fuel vapor locking tendency can be obtained only by the laboratory determination of the vapor-liquid ratio *vs.* temperature relationship.

A vapor-liquid ratio apparatus similar to that described by Campbell, Lovell, and Mulligan (*2*) was first used in these laboratories in 1936 for the evaluation of the vapor-locking tendencies of motor fuel blends. The information gained proved to be of considerable value when the manufacture of large quantities of high octane aviation spirit became a national necessity.

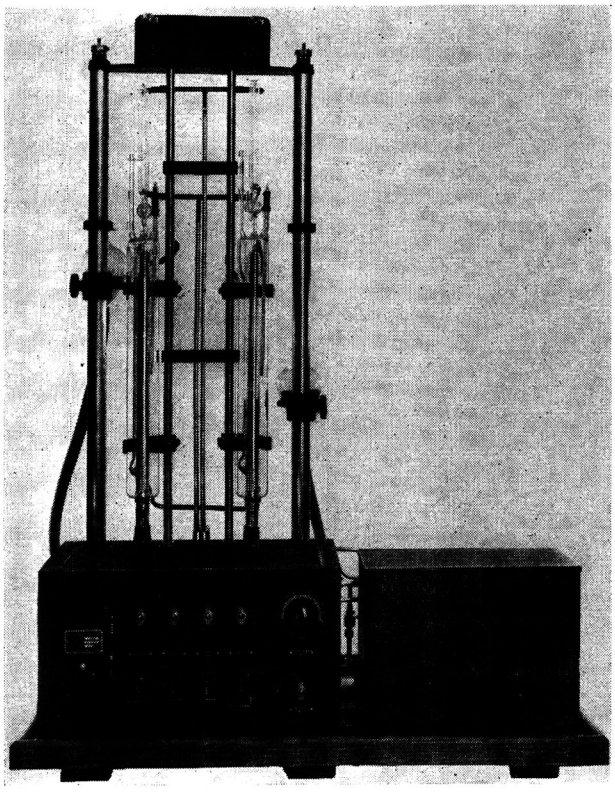

Figure 1. General view of apparatus

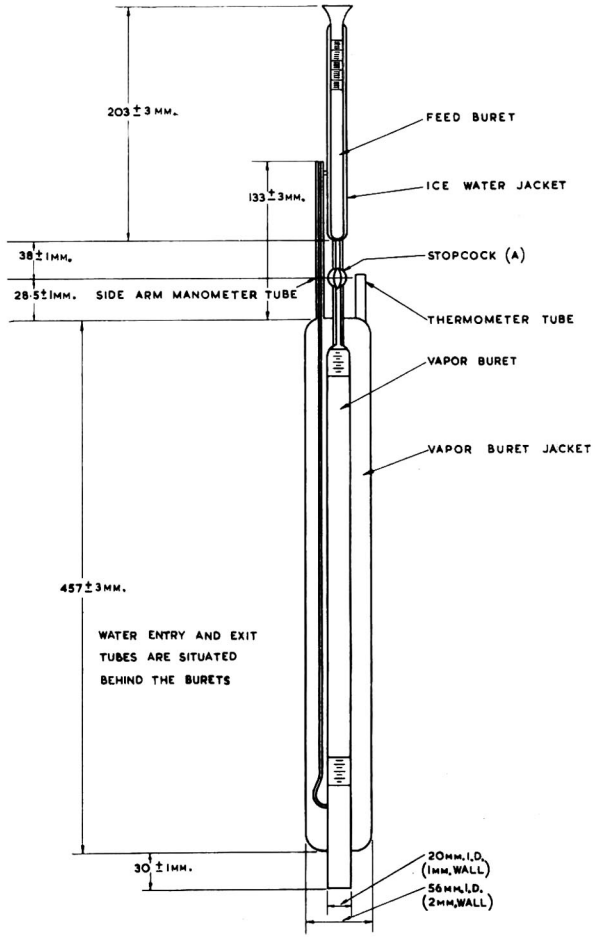

Figure 2. Vapor-liquid ratio buret

In connection with aircraft vapor lock difficulties during World War II, it became evident that a standard apparatus was needed to determine the vapor forming tendencies of fuels. Following the investigations of several vapor-liquid apparatus designs, the Coordinating Research Council, Inc., (CRC) adopted a modified version of that put forward by the Texas Co. and this now constitutes the CRC vapor-liquid ratio apparatus (*3*).

During 1949 the Campbell, Lovell, and Mulligan type of apparatus was modernized and made into a compact unit in the Sunbury laboratories. This equipment was preferred to that of the Coordinating Research Council, Inc., because of its smaller size and easier operation.

The Institute of Petroleum's volatility panel studied the apparatus and its method of operation and considered it desirable to ascertain what differences in results would occur between this method described and the CRC procedure. Preliminary information in this respect is provided by data supplied by the Shell Thornton Laboratories, which kindly agreed to carry out tests

with their modified CRC apparatus on a mixture of known chemical composition.

In this article, the Sunbury apparatus is described and details of its method of operation are provided. Experimental data are given which were obtained in order to assess the repeatability and reproducibility of the test.

EXPERIMENTAL

Apparatus and Method of Operation. A general view of the Sunbury vapor-liquid ratio apparatus is provided by Figure 1 and information on the more important components is to be found in Figures 2 to 6, inclusive.

Outline of Method. A measured volume of gasoline at 0° C. is introduced into a vapor buret and the volume of vapor in equilibrium with liquid, at a given temperature and at 760-mm. mercury pressure, is measured.

The ratio V/L is calculated and reported as the V/L ratio of the sample at the given temperature where

V = measured volume of vapor at the given temperature and L = measured volume of gasoline at 0° C. charged to the vapor buret

The amount of liquid in the vapor buret in equilibrium with the vapor cannot be measured accurately. It is therefore preferable to refer to the original charge of gasoline which is measured accurately at 0° C.

If desired, the V/L ratios at a series of temperatures over a suitable range—e.g., 100°–155° F.—may be determined and plotted in the form of a V/L-temperature curve, from which the temperatures for any desired V/L ratio may be read.

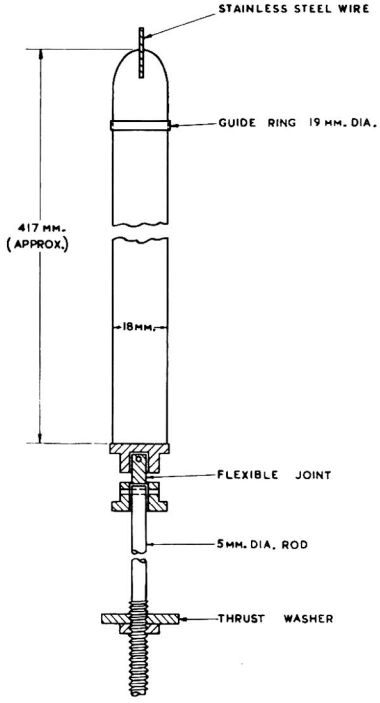

Figure 3. Vapor buret stirrer

Description of Apparatus. The apparatus consists essentially of a graduated glass tube or buret fitted with a glass jacket through which water at a controlled temperature flows for maintaining its temperature constant. Means are provided for introducing the sample and keeping it in a condition of constant agitation.

The eight essential component parts are described below and are illustrated in Figures 1 to 6 inclusive.

BURET ASSEMBLY. A jacketed feed buret, capacity of approximately 8 ml., around which ice-cold water can be passed and a jacketed vapor buret, capacity of 100 ml., around which water at any desired temperature can be pumped. The vapor buret is fitted with a side arm which serves as a manometer. The buret assembly is illustrated in Figure 2.

VAPOR-BURET STIRRER. A stainless steel blade (the length of which corresponds with that of the buret) shaped at the top to conform as near as possible with the shape of the buret neck, having a short length of stainless steel wire (approximately $^1/_{16}$ inch in diameter) attached to the tip and reaching to the extreme top of the vapor buret where it meets the stopcock. A guide ring is welded near the top of the blade and at the bottom is one half of a universal joint. The half joint is freely pinned to a short length of $^3/_{16}$-inch stainless steel rod rigidly attached to the second half joint. This, in turn, is freely pinned to the $^3/_{16}$-inch stainless steel drive shaft. The two pins referred to are at right angles to each other.

The stainless steel drive shaft passes down through a bearing and gland to a flexible coupling (see Figures 3 and 4).

STIRRER MOTOR. This stirrer motor is fitted with a reduction gearbox giving a speed of 300 r.p.m. The Klaxon, Type M.K.S. UJI-WI, is suitable for this purpose.

BEARING AND GLAND. The upper portion of the stainless steel gland has a shoulder formed on it and a spigot fitting freely into the base of the buret. The joint is made by a short length of rubber tubing over the glass, wired and bound with insulating tape to give reinforcement against the head of mercury.

The upper portion is screwed into the lower through a hole in the supporting platform, which is clamped between them. The lower portion under the platform is a grease gland and is fitted with an entry tube for mercury to pass into the vapor buret. The entry tube is fitted with rubber tubing connected to a mercury reservoir (see Figure 4).

FEED SYSTEM. To avoid loss by evaporation during the charging of the apparatus, a sealed feed system is employed. This consists of a 50-ml. bottle, fitted with a separating funnel acting as a mercury reservoir and a delivery tube for insertion into the cooled feed buret. The sample is delivered to the buret by mercury displacement (Figure 5).

COOLING SYSTEM. For circulating ice-cold water round the feed buret, a cooling system illustrated in Figure 6 is used. This consists of a copper coil fitted into a tank which can be filled with ice. The coil is connected to the main water supply at one end and to the feed buret jacket at the other. A drain tube is provided for the exit from the cooling jacket and for draining the ice tank.

HEATING SYSTEM. For circulating water at any desired temperature round the vapor buret, a heating system (Figure 6) is employed which consists of a lagged tank fitted with inlet and outlet tubes, an overflow tube to drain, and an additional inlet to allow displacement of hot water by mains cold water.

Heating is provided by a 1500-watt immersion heater controlled by means of a Sunvic regulator.

The inlet and outlet tubes are connected to the vapor buret jacket via a Stuart-Turner pump which circulates the tank water through the vapor buret jacket.

THERMOMETER. Thermometer, with a range −10° to +110° C., smallest subdivision 1° C., calibrated before use.

Calibration of Burets. Calibrate the feed and vapor burets and refer the subsequent experimental readings to the calibration curves.

In the case of the vapor buret, make allowance for the displacement due to the stirrer by weighing mercury into the buret, held in an inverted position, with the stirrer supported temporarily in its correct relative position in a cork. Remove the stopcock, seal one end of the bore with Sell-O-Tape, fill with mercury and weigh the contained mercury. Calculate the volume from the weights of mercury.

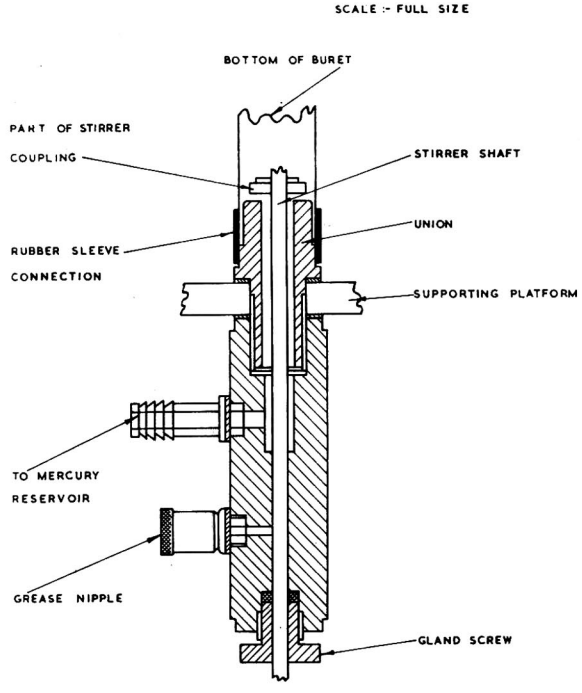

Figure 4. Bearing and gland

Preparation of Apparatus. Be sure that the taps (T_1 and T_3, Figure 6) in the cooling tank and the heating system drain lines are closed and that the tap in the water return line to the heating tank (T_2) is open.

Fit thermometers into their appropriate places in the vapor buret jackets using rubber tubing to keep them in place.

Fill the cooling tank with crushed ice.

Connect inlet 4 (inlet to heating tank) to the main laboratory water supply. Fill the heating tank with water (which should be at about 15° C.) until the water overflows through 3 (overflow from heating tank). Turn off the main water supply.

Connect inlet 5 (cooling coil inlet) to the main laboratory water supply. Turn on water so that there is a slow rate of flow through the cooling jackets of the feed burets as seen from the rate of flow from the drain tube 2 (drain from cooling system).

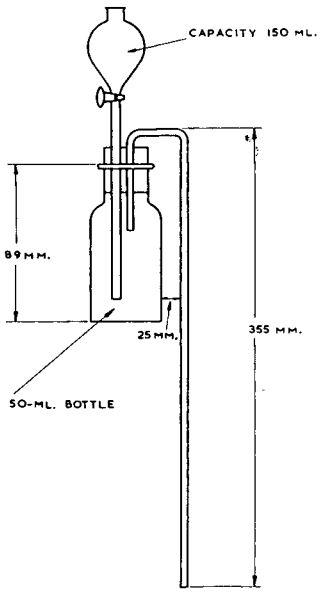

Figure 5. Feed system

Connect the electrical circuit to a main supply and switch on main switch.

Switch on circulating pump so that water is pumped from the heating tank through the vapor buret jackets and back to the heating tank. The thermometers should read approximately 15° C.

Check that the gland is filled with grease. Fill the mercury reservoir with clean, dry mercury and raise it to expel the air completely from the vapor buret by opening stopcock *A* (Figure 2). Adjust the mercury until the stopcock bore is just filled. Close the stopcock.

The apparatus is now ready for the introduction of the sample.

Procedure. Take the sample in a 50-ml. bottle of the type shown in Figure 5 and employed in the feed system. Precautions against the loss of light material in the sample must be taken by observing, as far as practicable, the procedure for sampling for vapor pressure tests (see ASTM D 323 or IP 69). Fill the bottle to just below the neck, stopper securely with a good quality cork, and keep in ice or in a refrigerator held at 0° ± 5° C. until required for test.

The following procedure refers to the preparation of a V/L temperature curve. If a value of V/L at a single temperature is required, this procedure must still be followed but vapor volume readings need not be taken until the desired temperature is reached.

Record the barometric pressure.

Remove the cork from the bottle containing the sample and replace by the separating funnel and delivery tube fitting shown in Figure 5. Put clean, dry mercury in the separating funnel and run in sufficient to displace all the air in the system and allow a little sample to run to waste. (Perform the above operations as quickly as possible, so that the sample does not warm up appreciably.)

Stand the sample bottle on the platform above the chilled feed buret and run in about 5 ml. of sample. Record the feed buret reading.

Open the vapor buret stopcock and lower the mercury reservoir of the vapor buret, thus drawing in a portion of the sample. For normal motor gasolines about 2 ml. is sufficient. Record the feed buret reading. Introduce a few drops of mercury into the feed buret to form a seal above the stopcock. Take the difference between this and the first reading, refer to the calibration chart for the feed buret, read the volume in milliliters, and deduct the volume of the stopcock bore. Record this result as the value of *L*.

Switch on the stirrer.

Lower the mercury reservoir so that the level of the mercury in the manometer side arm is approximately 10 mm. below that in the buret. This reduces the pressure slightly in the buret in order to assist in the removal of vapor from the solution and is particularly necessary during the heating periods.

When equilibrium is reached, switch off the stirrers and adjust the mercury reservoir so that the pressure in the vapor buret is 760 mm. If the barometric pressure is below 760 mm., raise the level of mercury in the manometer side arm so that it is above that in the buret by an amount equal to the difference between 760 mm. and the barometric pressure. If the barometric pressure is above 760 mm., lower the level of mercury in the manometer side arm so that it is below that in the buret by an amount equal to the difference between the barometric pressure and 760 mm.

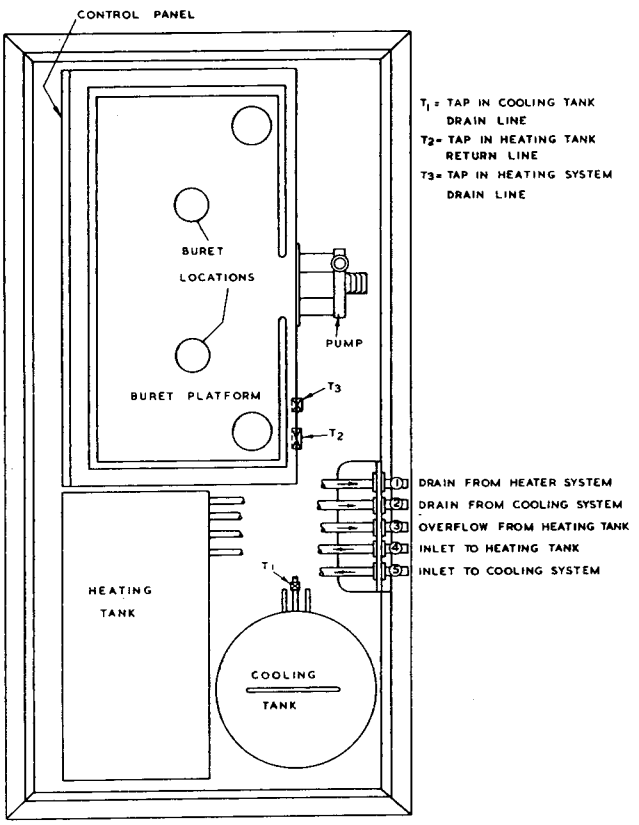

Figure 6. General arrangement of apparatus

Record the vapor buret reading, refer to the calibration chart, and read the vapor volume in milliliters. This is recorded as the value, *V*. If the volume of vapor is small, read the liquid volume and record the difference between this and the original charge as the volume of vapor.

Record the vapor buret temperature to the nearest 0.5° C.

Switch on the stirrers and the heating tank heater and raise the temperature by 3° to 4° C. Adjust by means of the temperature control.

Repeat operations starting with lowering of mercury reservoir and continue until sufficient number of points are available to plot a V/L *vs.* temperature curve.

Calculation and Reporting. Calculate the V/L ratio at each temperature. Plot in the form of a V/L *vs.* temperature curve, smoothing if necessary.

Present the results in the form of a table, prepared from the curve, giving V/L ratios for a series of temperatures. If necessary, the temperatures corresponding to specific V/L ratios may also be deduced from the curve.

Precision. The precision of this method has not yet been fully established.

Cleaning and Drying of Buret at Conclusion of Test. Expel liquid and vapor through the buret tap and remove by suction from the feed buret. A filter funnel trap connected to the vacuum supply is convenient for removing the sample.

Take a charge of acetone and wash out the buret by raising and lowering the mercury reservoir. Remove the acetone.

Lower the mercury level below the side-arm manometer tube level and draw air through the buret. Care must be taken to completely remove all traces of acetone.

Table I. Comparison of Vapor-Liquid Ratio Data

Test No.	Sunbury Results 1	2	3	4	Mean	Modified CRC Results 1	2	Mean	Theoretical Data
Temp., ° F.	Vapor-Liquid Ratios								
100	0.1	0.3	0.3	0.1	0.2				
110	0.5	0.5	0.6	0.4	0.5				
115	0.75	0.8	0.8	0.6	0.75				
120	1.0	0.9	1.0	0.8	0.9				
125	1.2	1.0	1.2	0.9	1.1				
130	1.5	1.2	1.5	1.3	1.4				
135	2.3	1.8	2.6	3.2	2.5				
140	5.2	4.1	5.0	6.7	5.25				
145	13.0	11.5	13.0	15.0	13.1	13.4	14.0	13.7	14.6
150	27.9	25.8	27.9	29.9	27.9	26.8	26.0	26.4	28.5
155	42.7	40.0	43.0	44.9	42.65	46.0	44.5	45.3	48.5
Vap.-Liquid Ratios	Temperatures, ° F.								
10	143.6	144.5	144.0	143.0	143.8	143.2	143.0	143.1	142.3
20	147.3	148.0	147.3	146.7	147.3	147.7	147.7	147.7	147.8
30	150.8	151.5	150.7	150.1	150.8	151.0	151.0	151.0	150.5
40	154.1	155.0	154.0	153.4	154.1	153.8	154.0	153.9	153.1

Cooling Water in Heating Tank at Conclusion of Test. Before commencing the next tests, if the water in the heating tank is still hot, carry out the following operations to displace it with cold water

Open tap T_1

Switch on circulating pump, to remove some of the water from the tank

Close T_1, and inject cold water from main supply through inlet 4 (inlet to heating tank) until water overflows through 3 (overflow from heating tank)

Circulate the warm water through the system to reduce its temperature

Repeat this cooling operation until the water is cold and the thermometer reads about 15° C.

RESULTS

In order to evaluate the repeatability and accuracy of the test, four determinations were made on a blend consisting of 30 parts by volume of normal pentane and 70 parts by volume of isooctane. The results of these tests are given in Table I, together with those obtained on the same blend using the modified CRC apparatus erected at the Thornton Laboratories. This apparatus differs from that of the Coordinating Research Council, Inc., in that the thermostat bath in which the burets are supported is directly heated and controlled instead of being fed from an auxiliary thermostat tank. The volumes of liquid and vapor in the buret are measured by determining the difference between the mercury and liquid levels and a reference pointer using a cathetometer and a traversing telescope. The burets are calibrated over a range of temperatures by withdrawing small quantities of mercury and measuring the drop in the mercury levels.

In both sets of results the vapor-liquid ratios were calculated on the liquid volume of the charge to the buret measured at 0° C. Normally, the CRC procedure requires measurement of the vapor and liquid volumes over a range of temperatures and the ratio of these measured volumes is reported as the V/L ratio. To compare with the Sunbury apparatus, however, the procedure described was adopted so that the results would be on a common basis.

Theoretical vapor-liquid ratio data for this blend are also shown in Table I. These were calculated by the method given in the CRC report (*3*). A modification is introduced to allow for reference to the volume of the original charge at 0° C., as used in the Sunbury method, instead of the volume of liquid at equilibrium with the vapor at the test temperature as used in the CRC calculation. The physical constants for the hydrocarbons used were taken from current authorities (*1, 4*).

Table II gives the results of vapor-liquid ratio determinations made in duplicate with the Sunbury apparatus on various samples of motor fuel blends.

In order to gain some preliminary information regarding the reproducibility of the method, samples of another gasoline blend were sent to three other laboratories for evaluation in the Sunbury-type equipment previously supplied to them. The results of these tests are listed in Table III.

DISCUSSION

The Sunbury apparatus for the determination of the vapor-liquid ratios of motor gasolines is adequately controlled, easy to operate, and yields results of sufficient precision for all practical purposes. The time required for a complete determination in duplicate is about 1.5 hours.

It has been used successfully at the Sunbury Research Station of The British Petroleum Co., Ltd., in connection with studies

Table II. Results of Vapor-Liquid Ratio Determinations

(Duplicate measurements with Sunbury apparatus on samples of motor fuel blends)

Sample	1		2		3		4		5		6		7	
Duplicates	a	b	a	b	a	b	a	b	a	b	a	b	a	b
Temperature, ° F.	Vapor-Liquid Ratios													
70	0.1	0.1	0.1	0.1										
75	0.2	0.2	0.2	0.2										
80	0.5	0.5	0.3	0.3	0.4	0.4	0.2	0.2	0.1	0.1	0.1	0.1	0.1	0.1
85	1.5	1.5	0.4	0.4	0.5	0.5	0.25	0.25	0.15	0.15	0.15	0.15	0.15	0.15
90	3.7	3.7	0.5	0.5	0.6	0.6	0.3	0.3	0.2	0.2	0.2	0.2	0.2	0.2
95	7.5	8.0	0.6	0.6	0.7	0.7	0.4	0.4	0.3	0.3	0.25	0.25	0.25	0.25
100	12.6	12.7	0.7	0.7	0.8	0.8	0.5	0.5	0.4	0.4	0.3	0.3	0.3	0.3
105	17.8	17.8	0.8	0.8	1.2	1.2	0.7	0.7	0.5	0.5	0.4	0.4	0.4	0.4
110	23.0	22.5	1.2	1.2	2.6	2.6	0.9	0.9	0.7	0.7	0.7	0.7	0.6	0.6
115	28.2	27.5	2.2	2.2	5.5	5.5	1.7	1.7	1.2	1.2	2.0	1.0	1.0	1.0
120	33.5	32.5	4.2	4.2	10.2	10.2	3.5	3.5	2.4	2.4	4.4	1.9	1.9	1.9
125	38.5	37.5	8.3	8.3	16.0	16.0	7.6	7.6	5.7	5.7	10.0	10.0	4.2	4.2
130	44.0	42.3	13.7	13.7	23.6	23.6	15.6	15.6	12.5	12.5	17.5	17.5	8.5	9.0
135	...	...	20.5	20.5	33.2	33.2	24.2	24.7	20.9	20.9	25.0	25.0	15.2	16.2
140	...	...	28.3	28.3	...	...	32.7	34.2	30.3	30.8	33.0	32.0	24.2	25.0
145	...	...	37.4	37.4	...	...	...	...	...	...	...	...	32.7	34.0
Vapor-Liquid Ratios	Temperatures, ° F.													
10	97	97	126.5	126.5	120	120	127	127	128	128	125	125	131.5	131
20	107	107	135	135	127.5	127.5	132.5	132.5	134.5	134.5	131.5	131.5	138	137
30	116.5	117	141	141	133.5	133.5	138	138	139.5	139.5	138	138.5	143.5	143
40	126.5	127	146	146	138	138	143.5	143	144.5	144.5	145	145.5	149	148.5

Table III. Comparison of Vapor-Liquid Ratio Results

Obtained in duplicate with similar apparatus at different laboratories for motor fuel sample 8

Laboratory Duplicates	K		L		G		S
	a	b	a	b	a	b	
Temperature, ° F.	Vapor-Liquid Ratios						
110	0.6	0.7	0.6	0.6	0.6	0.6	0.9
115	1.0	1.0	0.9	1.1	0.9	0.9	1.2
120	2.2	2.2	1.8	1.9	1.5	1.6	1.9
125	5.1	5.1	3.6	3.8	3.0	3.5	3.5
130	10.2	10.2	8.1	8.7	7.6	7.7	7.2
135	19.2	19.2	16.0	16.9	15.6	15.6	15.8
140	28.7	29.2	26.2	27.7	27.6	27.2	26.0
145	38.7	38.7	37.3	39.4	40.7	38.0	36.0
Vapor-Liquid Ratios	Temperatures, ° F.						
10	130	130	131.5	131	132	132	132
20	135.5	135.5	137	136.5	137	137	137
30	140.5	140.5	141.5	141	141	141.5	142
40	146	145.5	146	145	144.5	145.5	147

of vapor locking tendency (*5*). The results are considered to offer better criteria for the suitability of motor gasolines than the more usual volatility characteristics such as vapor pressure or distillation range.

From the results obtained at the Sunbury laboratories for the blend of 30 parts of *n*-pentane to 70 parts by volume of iso-octane (Table I) it will be seen that the variations in temperature from the mean values corresponding to the vapor-liquid ratios chosen are:

Vapor-liquid ratio	10	20	30	40
Variation from mean temperature, ° F.	+0.7, −0.8	0.6	0.7	0.9
		0.7	0.6	0.7

from which the average deviation from the mean is ±0.7° F.

Furthermore the data from the CRC apparatus in Table I compare favorably with the results obtained on the Sunbury apparatus. The deviation from the mean Sunbury values varies between +0.4° and −0.8° F.

When considering the duplicate results on motor fuel blends (Table II), the maximum temperature differences between any pair of results for vapor-liquid ratios of 10 to 40 are 1° F. which may be expressed as ±0.5° F. This order of repeatability (±0.5° F.) is satisfactory and is confirmed by the duplicate results reported from three other laboratories (Table III). The data at present available for the assessment of the reproducibility of the method are somewhat meager and are limited to those presented in Table III. The maximum temperature difference at any of the vapor-liquid ratios quoted—viz., 10, 20, 30, and 40—is 2° F. and may be interpreted tentatively as ±1° F. However, further trials of the procedure at different laboratories are necessary before this figure for reproducibility may be substantiated.

The satisfactory agreement of the experimental with the calculated theoretical data on the blend of 30 parts of *n*-pentane to 70 parts by volume of iso-octane (Table I) further indicates the suitability of the apparatus for the determination of vapor-liquid ratio data.

LITERATURE CITED

(1) Am. Petroleum Inst., API Project 44, "Selected Values of Properties of Hydrocarbons and Related Compounds," data sheets d-E, k, k-E.
(2) Campbell, J. M., Lovell, W. G., and Mulligan, M. J., *J. Soc. Automotive Engrs. Trans.* 39, No. 3, 356 (September 1936).
(3) Coordinating Research Council, "Evaluation of the CRC *V/L* Apparatus," report issued January 1951, rev. March 1953.
(4) Inst. Petroleum (London), "Standard Methods for Testing Petroleum and Its Products," 14th ed., method 59/55, Table V, 1955.
(5) Sheppard, G. F., and Withers, J. G., "Assessment of Car and Fuel Factors Affecting Vapor Locking Tendency," Inst. Mech. Engrs. (London), preprint (January 1955).

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Assay of Picric Acid by Coulometry at Controlled Potential

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At a mercury cathode whose potential is kept constant at −0.40 volt *vs.* S.C.E., the reduction of picric acid from hydrochloric acid solutions proceeds rapidly and quantitatively under the proper conditions to 2,4,6-triaminophenol. Integrating the current which flows during such an electrolysis is recommended for the assay of picric acid in conjunction with a conventional alkalimetric titration.

AN ACCURATE evaluation of the purity of picric acid by classical techniques is so far from being a simple matter that no entirely satisfactory procedure for the assay of this reagent appears ever to have been proposed. Since lower nitrated phenols are certain to be present as contaminants, it is evident that the common alkalimetric "assay" procedure necessarily gives a fictiously high value of the picric acid content. Moreover, the fact that thoroughly dry picric acid is a somewhat hazardous substance, so that the assay is preferably carried out on a wet sample, makes it difficult to set up a standard by which the result can be judged.

In this paper there is proposed a procedure which involves two independent analyses of an undried sample. One consists of the measurement of the amount of current consumed in the quantitative electroreduction of the organic nitro groups by the electrolysis of a hydrochloric acid solution of the sample at a mercury cathode, whose potential is kept constant at a suitable value. This gives the number of equivalents of reducible material per gram of sample. The other analysis consists of a conventional alkalimetric titration, which gives the total number of equivalents of replaceable hydrogen per gram of sample. As is shown, the reduction of one mole of picric acid, containing one equivalent of replaceable hydrogen, consumes 18 faradays of electricity. Consequently, the ratio between the coulometric and alkalimetric values is exactly 18 for picric acid containing water as the only impurity. As this ratio is 12 for any dinitrophenol present, its value serves as a measure of the relative amounts of tri- and dinitrophenol present.

The electrolytic reduction of picric acid from hydrochloric acid solutions at a mercury cathode was first studied by Lingane (*3, 4*). Employing a 0.4m*M* solution of picric acid in 0.1*M* hydrochloric acid, Lingane found $n = 17.1$, a value which Bergman and James, using identical conditions, later duplicated (*1*). On this basis Lingane (*4*) suggested that the reduction product is bis-(3,5-diamino-4-hydroxyphenyl)-hydrazine:

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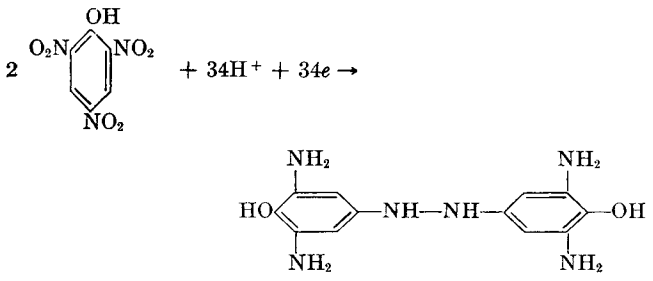


Kolthoff and Lingane in collaboration with Wawzonek (*2*) discussed the possibility that this assumed product might undergo the benzidine rearrangement, while Müller (*6*) attempted to explain the polarograms of picrate solutions at various pH values on the basis of an over-all 17-electron reduction.

Pearson (*8*) and Neiman, Kuznetsov, Rabinovitch, and Ryabov (*7*) also studied the polarographic characteristics of picric acid at various pH values. Pearson stated that the total diffusion current corresponded approximately to a 16-electron reduction, assuming that picrate and benzoate ions have equal diffusion coefficients. In view of the rather large differences between the sizes and formula weights of these ions, this is very unlikely to be the case.

Herein it is shown that the reduction of picric acid consumes exactly 18 faradays per mole (and must therefore give 2,4,6-triaminophenol) provided that the concentration of picric acid is very low and the acidity is relatively high. Otherwise—e.g., above 0.2m*M* picric acid in 0.1*M* hydrochloric acid, above 1m*M* picric acid in 1*M* hydrochloric acid, or above 1.5m*M* picric acid in 3*M* hydrochloric acid—the value of n falls below 18. This is evidently the result of a side reaction which involves some intermediate reduction product: This reaction is favored by a high concentration of the intermediate (or, naturally, of the original picric acid), and is retarded by a high concentration of hydrogen ion. With 0.4m*M* picric acid in 0.1*M* hydrochloric acid, the authors find, in agreement with Lingane and with Bergman and James, that the apparent value of n is close to 17. At still higher concentrations of picric acid, however, n becomes distinctly less than 17. This may be interpreted to mean that the reduction of picric acid, like that of nitrobenzene, proceeds via a substituted hydroxylamine, which can then rearrange to form an aminophenol (in this case, a dihydroxytriaminobenzene). Values of n then can be found lying between 16 and 18, depending on the relative fractions of the hydroxylamine undergoing rearrangement and reduction.

EXPERIMENTAL

The potentiostat and current integrator used in this work were manufactured by Analytical Instruments, Inc., Bristol, Conn., and have been described (*5*). All electrolyses were carried out in a double diaphragm cell (*5*) filled throughout with hydrochloric acid of the same concentration. An efficiently stirred mercury pool served as the working electrode; the auxiliary electrode was a helix of stout platinum wire. A few drops of saturated hydrazine dihydrochloride were added to the auxiliary electrode compartment to serve as a depolarizer.

A sample of pure picric acid was secured by recrystallizing the reagent grade chemical once from water, once from 95% ethyl alcohol, and finally again from water. A small portion of the product was dried for several weeks over anhydrous magnesium perchlorate, and used to prepare an aqueous solution of exactly known concentration. All other chemicals used (excepting one sample of technical grade picric acid) were ordinary reagent grade and were not further purified. The carbonate-free sodium hydroxide solution was standardized against primary standard grade potassium hydrogen phthalate.

All weights and volumetric apparatus had been carefully calibrated by conventional techniques.

To begin a coulometric determination, the central and auxiliary electrode compartments of the cell were filled with hydrochloric acid of the desired concentration. About 75 ml. of acid was then added to the working electrode compartment, followed by the desired volume of picric acid solution. The solution in the working electrode compartment was then completely deaerated by bubbling a rapid stream of prepurified nitrogen through it for not less than 15 minutes.

Complete removal of oxygen from the solution is more important here than in most coulometric analyses. Normally, the interference of oxygen results from the fact that it is reduced at the working electrode together with the substances being determined. As this reduction proceeds at a finite rate, one can usually be content with removing most, but not all, of the dissolved oxygen before beginning the electrolysis. Ordinarily only a small fraction of the oxygen left will be reduced, whereas most of it will be removed by the stream of nitrogen. This is not true, however, in the picric acid reduction, for the reduction product formed at the very beginning of the electrolysis is reoxidized almost instantaneously by dissolved oxygen, the entire amount of which is thus rapidly reduced, giving a positive error. Since, as is shown, only very small amounts of picric acid can be handled in the coulometric procedure, the relative error thus incurred can be very serious indeed.

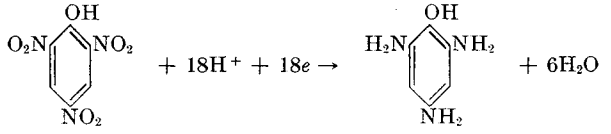
When the deaeration is complete, 25 ml. of mercury is added to the working electrode compartment, the potentiostat is adjusted to maintain the working electrode potential ($E_{w.e.}$) at −0.40 volt *vs.* S.C.E., and the electrolysis is begun.

In these experiments, the total volume of the solution in the working electrode compartment was always close to 80 ml., and in consequence the electrolyses proceeded more rapidly than with the larger volumes of solution used in earlier work (*5*). Every electrolysis was complete—i.e., the register on the current integrator came to a full stop—within 25 to 30 minutes. The solution must be discarded when the electrolysis is complete; addition of another aliquot of the sample in an attempt to run a check determination invariably leads to air oxidation and high results.

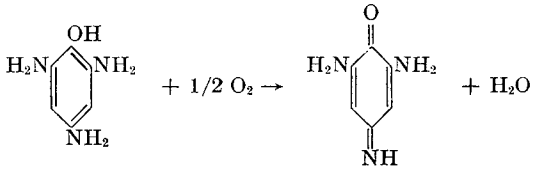
All of the electrolyses were carried out with the integrator set on its 1 microfaraday per count range, and the number of counts recorded was multiplied by the previously determined (*5*) calibration factor 1.00082, to secure the number of microfaradays used.

DATA AND DISCUSSION

A polarogram of picric acid in 1*M* hydrochloric acid consists of a single irreversible wave rising from zero applied electromotive force and having an extremely well defined plateau. When dilute solutions of picric acid were electrolyzed at −0.40 volt *vs.* S.C.E., the data shown in Table I were secured. These data show conclusively that the reduction of picric acid under these conditions is an 18-electron process, and yields 2,4,6-triaminophenol as the product:



The electrolysis of a dilute picric acid solution finally yields a perfectly colorless solution, which becomes yellow brown almost instantly when exposed to air, as a result of the reaction



As might be expected on the basis of the large number of electrons involved, the course of the reaction is very complex. The initially yellow solution becomes successively green, brown, pink, and, at about 98% completion, a light purple which fades slowly

Table I. Determination of *n* for Picric Acid

(Known amounts of picric acid were added to 75-ml. portions of hydrochloric acid, and the deaerated mixtures were electrolyzed at a mercury cathode whose potential was maintained at −0.40 volt *vs.* S.C.E.)

Micromoles of Picric Acid Taken	HCl, *M*	Microfaradays Consumed	*n* Faradays/ Mole
7.58	0.1	136.6	18.02
15.14	0.1	272.5	18.00
30.22	1	545.0	18.03
59.87	1	1079.6	18.03
75.34	1	1353.6	17.97
		1356.1	18.00
		1356.1	18.00
		1356.1	18.00
90.33	1	1624.6	17.99
		1626.6	18.01
90.67	3	1631.8	18.00
151.3	3	2722.4	17.99
		2722.6	17.99

Mean 18.00 ± 0.01$_6$ (std. dev.)

until the electrolysis is complete. It is not known whether these intermediates result from a stepwise reduction or from reactions between the electrolysis product and unreduced picric acid.

Effect of Working Electrode Potential. When solutions of 34.88 micromoles of picric acid in 75 ml. of 1*M* hydrochloric acid were electrolyzed at various potentials, the data shown in Table II were secured. The values secured at potentials between −0.20 and −0.60 volt are in good agreement with the expected value, but at more negative potentials hydrogen ion begins to be reduced along with the picric acid, and high results are obtained. A working electrode potential of −0.40 volt *vs.* S.C.E. is therefore recommended.

Table II. Effect of Working Electrode Potential on Electrolytic Reduction of Picric Acid

Working Electrode Potential, Volt *vs.* S.C.E.	Microfaradays Required		Error, %
	Calcd.	Exptl.	
−0.20	627.8	627.7	−0.02
		628.2	+0.06
−0.40		627.8	±0.00
		628.0	+0.03
		625.5	−0.37
		627.9	+0.02
−0.60		627.6	−0.03
		627.6	−0.03
−0.70		629.2	+0.22
		628.5	+0.11
−0.80		636.5	+1.4
		634.5	+0.9
−0.90		659.5	+5.1

Effect of Hydrochloric Acid Concentration. A number of solutions of 56.01 micromoles of picric acid in hydrochloric acid media of various concentrations were electrolyzed at −0.40 volt *vs.* S.C.E., with the results shown in Table III. These data show that accurate results are obtainable at any hydrochloric acid concentration between 0.3 and 3*M*. In 6*M* acid the values are high, and reducing the working electrode potential to −0.20 volt *vs.* S.C.E. decreases but does not entirely eliminate the error. In any case, no advantage would seem to result from the use of a hydrochloric acid concentration differing from 1*M*. As seen in the following paragraph, the hydrochloric acid concentration and picric acid concentration both exert a profound influence on the results obtained with all but the most dilute picric acid solutions.

Effect of Picric Acid Concentration. The results shown in Table IV were secured by adding known volumes (10 ml. or less) of a stock solution of picric acid to 75 ml. of hydrochloric acid, and electrolyzing the mixture at −0.40 volt *vs.* S.C.E. According to these data, accurate results can be secured only when the initial concentration of picric acid in a 1*M* hydrochloric acid solution is less than about 1m*M*; the electrolysis of a more concentrated solution yields an orange solution, and the quantity of electricity consumed is abnormally low. This orange product cannot be reduced at any potential before the discharge of hydrogen ion begins. The orange product is no doubt formed by the interaction of the products of the primary reaction at the electrode surface. Except for the picric acid concentration at which the negative error becomes appreciable, the general trends in 0.1 and 3*M* hydrochloric acid are very similar to that in 1*M* acid. The nonelectrolytic side reaction is evidently retarded by increasing acid concentration.

Table III. Effect of Hydrochloric Acid Concentration on Electrolytic Reduction of Picric Acid

[HCl], *M*	Micromoles of Picric Acid		Error, %
	Taken	Found	
0.3	56.01	56.05	+0.07
		56.09	+0.14
0.5		55.91	−0.18
1.0		55.99	−0.04
		56.09	+0.14
		56.03	+0.04
2.0		56.10	+0.16
		55.94	−0.13
3.0		56.00	−0.02
		55.89	−0.21
6.0		56.39	+0.68
		56.57	+1.00

Analyses of Commercial Samples of Picric Acid. Two commercial samples of picric acid were analyzed by the proposed procedure. The wet material was thoroughly mixed, and a 1.5-gram sample was weighed out, transferred to a 250-ml. volumetric flask, dissolved in recently boiled water, and diluted to the mark. Two 100-ml. portions of this solution were titrated with standard sodium hydroxide. In the interest of securing greater

Table IV. Coulometric Determination of Various Amounts of Picric Acid in Hydrochloric Acid

Micromoles Picric Acid Taken	Picric Acid Concn., m*M*	Micromoles Picric Acid Found	Error, %
	0.1*M* HCl		
7.58	0.10	7.59	+0.12
15.14	0.20	15.14	±0.00
22.72	0.29	22.39	−1.5
30.22	0.38	28.69	−5.1
59.87	0.72	54.84	−8.4
	1*M* HCl		
13.88	0.18	13.89	+0.10
28.03	0.36	28.09	+0.25
		28.04	+0.07
56.01	0.71	56.01	−0.01
		55.89	−0.21
69.68	0.87	69.79	+0.16
		69.89	+0.30
		69.85	+0.24
		69.71	+0.04
101.1	1.23	100.8	−0.31
		100.7	−0.39
139.6	1.64	138.5	−0.79
		138.7	−0.64
172.7	2.16	160.9	−6.8
	3*M* HCl		
15.14	0.20	15.16	+0.15
30.22	0.38	30.25	+0.13
59.87	0.72	59.81	−0.10
90.67	1.04	90.66	−0.10
151.3	1.59	151.2	−0.06
		151.2	−0.05
172.7	2.16	170.3	−1.4
226.0	2.83	219.8	−2.7

accuracy, a Beckman Model G pH meter was used to locate the equivalence point of the titration. Meanwhile a 25-ml. aliquot of the solution was diluted to 250 ml. with 1*M* hydrochloric acid in another volumetric flask, and two 25-ml. aliquots of this were analyzed coulometrically.

When a 1.5052-gram sample of reagent grade picric acid (containing, according to its manufacturer, 10.7% water) was analyzed by this procedure, it was found that 5.866 ± 0.004 meq. of base would have been required to neutralize the entire sample, and that 105.42 ± 0.09 millifaraday would have been required to reduce it. Assuming that the sample contained x millimoles of picric acid, for which $n = 18$, and y millimoles of dinitrophenol, for which $n = 12$, these values give

$$x + y = 5.866$$

and

$$18x + 12y = 105.42$$

whence the weight of picric acid in the sample is 1.338 grams (88.9%), and that of dinitrophenol is 5.2 mg. (0.35%). If water is the only other constituent of the sample, it would therefore be present to the extent of 10.75%, in good agreement with the direct determination.

A sample of technical grade picric acid analyzed in the same way was found to contain 83.4% picric acid and 3.1% dinitrophenols. It is evident that a simple alkalimetric titration of this material would give a seriously erroneous estimate of its picric acid content.

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LITERATURE CITED

(1) Bergman, I., and James, J. C., *Trans. Faraday Soc.* **50**, 60 (1954).
(2) Kolthoff, I. M., and Lingane, J. J., "Polarography." vol. **2**, p. 627, Interscience, New York, 1952.
(3) Lingane, J. J., "Electroanalytical Chemistry," p. 369, Interscience, New York, 1953.
(4) Lingane, J. J., *J. Am. Chem. Soc.* **67**, 1916 (1945).
(5) Meites, L., ANAL. CHEM. **27**, 1116 (1955).
(6) Müller, O. H., in "Physical Methods of Organic Chemistry" (A. Weissberger, editor), vol. **2**, pp. 1845–6, Interscience, New York, 1949.
(7) Neiman, M. B., Kuznetsov, L. I., Rabinovitch, I. B., and Ryabov, A. V., *Zavodskaya Lab.* **15**, 1280 (1949).
(8) Pearson, J., *Trans. Faraday Soc.* **44**, 683 (1948).

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Determination of Hydrogen in Titanium and Titanium Alloys

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As hydrogen in titanium and titanium alloys is considered an undesirable impurity, its determination is a matter of importance. In this paper an ignition method is proposed. The sample is ignited in oxygen in the presence of lead as a flux, the gases are passed through hot copper oxide to ensure complete oxidation of the hydrogen to water, and the water is collected in an Anhydrone collection tube. An important problem in developing the method was to find means for preventing the large amount of heat generated by the reaction between the titanium and oxygen from cracking the reaction tube. This problem was solved by weighing the sample in a piece of 96% silica tubing and resting this tubing in the reaction tube on clay supports.

THE effect of hydrogen on titanium is not as marked as the effect of oxygen and nitrogen on titanium, in so far as such properties as hardness, ductility, and electrical conductivity are concerned (*22, 28*). Hydrogen, however, can change the grain structure of titanium (*5, 12, 29*) and has a profound effect on notch bar toughness (*30, 34*). The effect of hydrogen that might be picked up during welding, forging, or pickling operations is controversial (*26, 35, 59*). No beneficial effect of hydrogen on titanium has been noted; therefore hydrogen must be classified as a contaminant and closely controlled (*34*).

For proper understanding of the problems involved in the determination of hydrogen in a metal it is essential to consider the metallurgical relationships between hydrogen and the metal. For no other element is there such a close tie-in between metallurgy and analysis. Titanium belongs to a group of metals, which includes zirconium, cerium, tantalum, and niobium, that is capable of absorbing considerable hydrogen in the solid state (*15, 21, 53, 54*). The solution of hydrogen in titanium is exothermic, and the solubility decreases as the temperature increases, the decrease becoming rapid at about 600° C. It is not possible to absorb much hydrogen in titanium at room temperature. However, if titanium is heated to about 300° to 400° C. and cooled to room temperature, the hydrogen that has been absorbed is retained. When hydrogen dissolves in titanium there is no film formation, as with oxygen or nitrogen (*24, 25*).

Hydrogen in titanium, when present in moderate amounts, is stable under a vacuum of 10^{-6} mm. of mercury to approximately 275° C. (*25*). The properties of titanium saturated with hydrogen (titanium hydride, TiH_2) (*1–3, 20*) have bearing on the determination of hydrogen in titanium. Titanium hydride contains about 4% hydrogen, is metallic in appearance, and is indefinitely stable at room temperature. When titanium hydride is heated in air, no change takes place until its temperature of dissociation is reached. The hydrogen is gradually expelled and burns with a quiet flame, and when most of the hydrogen has been expelled, the titanium starts to burn with considerable heat.

It is important to contrast the behavior of hydrogen in titanium with its behavior in iron, nickel, copper, aluminum, silver, molybdenum, and tungsten (*15, 53, 54*) in considering application of published methods for the determination of hydrogen in these metals to its determination in titanium. These metals can absorb only small amounts of hydrogen, and the hydride formed is not stable under ordinary conditions of temperature and pressure. The hydrogen is absorbed interstitially as atoms without specific interaction with the metal atoms. The process of solution is endothermic, and the solubility increases as the temperature is raised. When iron is cooled from a high temperature, the hydrogen is slowly evolved over a period of weeks and finally approaches the solubility of hydrogen in iron at room temperature. In determining the hydrogen content of freshly cast iron and steel specimens, such treatments as covering with

mercury, coating with metallic tin, or cooling in solid carbon dioxide or liquid air are necessary to prevent escape of the hydrogen (*62*, *64*). Such treatments are not necessary for titanium or titanium alloys.

In studying the hydrogen-titanium system, the hydrogen content of the material was determined by the pressure of the hydrogen or by means of a microbalance in a vacuum system. The vacuum fusion method has been the only available published method for the actual determination of hydrogen in titanium and titanium alloys. Several investigators have indicated that hydrogen in titanium and titanium alloys can be determined by vacuum fusion at the same time that oxygen is determined (*32*, *39*, *52*, *61*, *67*). As the vacuum fusion method is costly and time-consuming, an investigation was undertaken to develop an inexpensive rapid method.

Hydrogen has been determined in steels, bronzes, and nickel silver by drilling the specimen under water and collecting the hydrogen evolved (*7*, *38*, *41*), or by subjecting the specimens to cold work and collecting the hydrogen evolved (*6*, *8*, *57*). Such methods are not applicable to titanium, because the hydrogen in the metal is held too strongly. Ionic bombardment methods have been used for the determination of hydrogen in aluminum (*37*, *48*), iron (*37*), magnesium (*4*, *9*), tantalum (*43*), and palladium (*43*). The specimen is made the cathode in a vacuum discharge tube. Bombardment of the cathode with mercury ions induces an ionization of the gas atoms within the metal, and the ionized gases are then drawn from the metal by the high potential between the electrodes. The discharge tube is evacuated continuously, and the gases are collected for analysis. The equipment was not available in this laboratory to test the applicability of the ionic bombardment method to the determination of hydrogen in titanium.

Chemical solution methods, whereby the hydrogen in the metal is set free after solution of the sample, have been proposed. Hydrogen in aluminum was determined by dissolving the metal in bromine under carbon disulfide (*10*). The liberated hydrogen bromide was estimated gravimetrically as silver bromide, and the residual hydrogen was collected and oxidized with copper oxide. Hydrogen in steels has been determined after dissolving the sample in potassium cuprochloride solution (*23*). Solvents such as bromine and potassium cuprochloride will not dissolve titanium. A method has been proposed for determining hydrogen in titanium hydride by dissolving the material in 10% hydrochloric acid in the presence of dioxane and collecting the hydrogen evolved (*3*). This method would be inaccurate for small amounts of hydrogen in titanium.

Hydrogen has been determined in aluminum by fusion of the sample with a mixture of lead dioxide and ferrous oxide and measurement of the water formed (*63*). It is not known whether the method would be applicable to titanium. The hydrogen content of copper and aluminum has been determined by measuring the relative number and size of pinholes or blisters, or the density of the specimens (*13*, *16*, *17*, *56*). None of the methods depending upon blister or pinhole count or density are applicable to titanium because of the mode of combination of hydrogen in titanium. The method for determining hydrogen in aluminum by supersonic waves (*46*) or x-rays (*16*) is not applicable to the determination of hydrogen in titanium for the same reason.

Hydrogen has been determined in steels (*14*, *27*, *40*, *44*, *47*, *58*, *68*), magnesium (*9*, *33*), and zirconium (*31*) by hot vacuum extraction. In this procedure the sample is heated (but not melted) under a vacuum and the resultant gases are analyzed. Pepkowitz and Proud (*42*) have proposed a procedure for hydrogen in metals in which the sample is heated in an iron tube, the hydrogen is diffused through the tube under a vacuum, and the gases are analyzed. The hot vacuum extraction method and the method of Pepkowitz and Proud are applicable to titanium and titanium alloys. Hydrogen has been determined in steel by heating in an inert atmosphere and measuring the hydrogen released (*45*). This method does not seem applicable to titanium, since when a sample of titanium was melted in helium the hydrogen content of the material decreased only from 0.010 to 0.004% (*34*).

Hydrogen has been determined in aluminum (*55*) and magnesium (*9*) by passing a stream of inert gas through the molten metal and analyzing the gases released. Whether these methods could be applied to titanium is not known. Hydrogen has been determined in steels by measuring the thermal conductivity (*51*). The sample is fused under a vacuum, and the thermal conductivity of the released gases is measured. The method depends upon the fact that the thermal conductivity of hydrogen is seven times that of carbon monoxide or nitrogen. This method might be applicable to titanium or titanium alloys.

Hydrogen has been determined in steels (*18*, *19*, *36*, *49*, *50*, *60*, *65*, *66*) and magnesium alloys (*9*) by burning the sample in oxygen and collecting the water formed in a weighed bulb containing Anhydrone. Sometimes copper oxide (*9*, *36*), platinum gauze (*50*), or palladiumized asbestos (*50*) is used to ensure complete oxidation of the hydrogen to water. The ignition method was found to be applicable to the determination of hydrogen in titanium and titanium alloys. However, it was necessary to use lead as a flux to ensure complete recovery of the hydrogen.

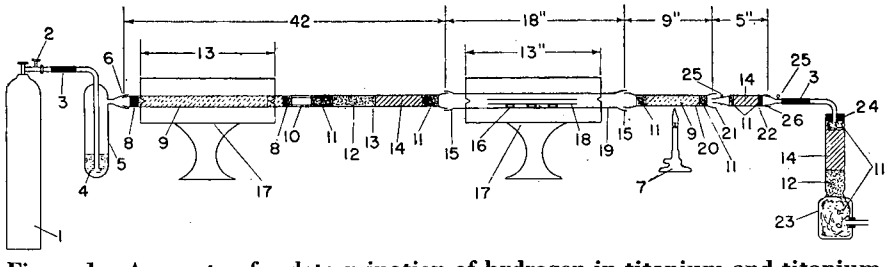

Figure 1. Apparatus for determination of hydrogen in titanium and titanium alloys

1. Tank of oxygen
2. Needle valve
3. Tygon tubing
4. Sulfuric acid
5. Small gas-washing bottle
6. Ground-glass joint (24/40)
7. Meker burner
8. Crumpled ball of fine copper wire
9. Copper oxide
10. Small glass tube inserted to separate copper oxide from Ascarite
11. Glass wool
12. Ascarite
13. Purification tube (Vycor, 25 mm. in diameter)
14. Anhydrone
15. Ground-glass joint (29/42)
16. Clay supports
17. Electric furnace (hinge type)
18. Sample tube (Vycor, 15 mm. in diameter, 7 inches long)
19. Reaction tube (Vycor, 25 mm. in diameter)
20. Copper oxide tube (Vycor, 25 mm. in diameter)
21. Ground glass joint (10/30)
22. Collection tube (borosilicate glass, 15 mm. in diameter)
23. Calcium chloride tower (10 inches high)
24. Rubber stopper
25. Microstopcock
26. Ground glass joint (12/18)

APPARATUS AND MATERIALS

The apparatus used, together with its important dimensions, is shown in Figure 1. All the ground-glass joints shown in Figure 1 are held together by springs. A considerable problem in applying the ignition method to titanium and its alloys was to find means for preventing the large amount of heat generated by the reaction between titanium and oxygen from cracking the reaction tube. This problem was solved by weighing the sample in a piece of 96% silica tubing, 15 mm. in diameter and 7 inches long, and resting the tubing in the reaction tube on clay supports (three rounded clay covers for combustion boats, Leco). Probably an equally effective and cheaper method for preventing the cracking of the reaction tube would be to weigh the sample in a clay boat and insert this boat into a hollow cylinder made of clay or magnesia. The cylinder would then be inserted into the reaction tube

Table I. Results of Hydrogen in Standard Samples of Titanium and Titanium Alloys

Sample No.	Type of Alloy	Alloying Elements	Hydrogen, % Present, vacuum fusion	Hydrogen, % Found, proposed method	Hydrogen, % Average found, proposed method
WA2	Cr-Al-Ti alloy	5% Cr, 3% Al, 0.7% C	0.016	0.013 0.017	0.0150
WA9	RC-130B	4% Al, 4% Mn	0.005	0.006 0.007	0.0065
WA10	Comm. pure Ti	.....	0.006	0.007 0.007	0.0070
WA12	Ti-150A	2.7% Cr, 1.3% Fe	0.013	0.013 0.011	0.0120
RR3	....	.....	0.013	0.012 0.013 0.012	0.0123
RR4	....	.....	0.014	0.016 0.014 0.012	0.0140
RR5	....	.....	0.008	0.010 0.011 0.009	0.0100
RR6	....	.....	0.004	0.004 0.005 0.005	0.0047
RR7	....	.....	0.009	0.010 0.009 0.011	0.0100
RR8	...	.....	0.011	0.012 0.011 0.011	0.0113
RR12	....	.....	0.027	0.027 0.027 0.023	0.0257

(without clay supports). The authors were unable to obtain boats and cylinders of suitable dimensions. The reaction tube used in the apparatus can be made of either 96% silica or quartz. Following the reaction tube is a small tube containing copper oxide which is heated with a Meker burner (or a heater). The purpose of this tube is to ensure oxidation of the hydrogen to water. The water is collected in a small Anhydrone collection tube with stopcocks at each end. A protective tower containing Anhydrone and Ascarite is placed after the collection tube. The oxygen used in the determinations is purified by passing it through a long column of copper oxide heated to 750° C. and then through Ascarite and Anhydrone. The two furnaces used in the method should be of the hinge type (multiple unit, Hevi Duty Electric Co., Milwaukee, Wis.) because they should be removable. The furnace used for heating the copper oxide in the purification tube is regulated by a Potentiostat, while the furnace used for heating the reaction tube is kept at the maximum temperature obtainable (about 900° C.).

The blank for the method (using 8 grams of lead) was found to be insignificant (less than 0.1 mg.). In running a blank it was necessary to use a sample tube with its ends turned up in order to prevent the molten lead from flowing down the reaction tube and cracking it. Drillings, chips, and pieces can be used. Pieces are preferable. 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. The time required for a single determination is about a half hour. The apparatus is inexpensive and is not subject to breakdowns.

PROCEDURE

Ignite several pieces of 96% silica tubing, 15 mm. in diameter and 7 inches long, and several clay supports in a muffle at 1100° C. for 2 hours, remove, and store in a desiccator containing Anhydrone.

Turn on the electric heaters and heat the copper oxide in the purification tube to about 750° C. and the reaction tube containing three of the clay supports to about 900° C. while slowly passing oxygen through the system. Adjust the flow of oxygen to a moderate rate, connect the copper oxide tube (20 in Figure 1), and heat it with a Meker burner at full heat. The Meker burner should be placed about 2 inches from the inlet end of the copper oxide tube. Connect the collection tube and allow the oxygen to flow through it for a few minutes. Detach the collection tube, allow it to stand 5 minutes in the balance case, open momentarily to the atmosphere to equalize the pressure, and weigh. While continuing the flow of oxygen, raise the reaction tube about an inch, and remove the furnace. Allow the reaction tube to cool to below 300° C. but keep the furnace at about 900° C.

Prepare the sample in the form of drillings, chips, and pieces, clean twice with carbon tetrachloride, and dry in an oven at 70° C. Weigh the sample. Use 5 grams when the hydrogen content is less than 0.3% and 1 gram when the hydrogen content is 0.3 to 1%. Mix the sample with granulated lead (Mallinckrodt or Merck, reagent grade). Use 8 grams of lead for 5 grams of titanium and 2 grams of lead for 1 gram of titanium. Pour the mixture of the sample and lead into a piece of the 15-mm. 96% silica tubing and push the mixture to the middle 4 inches of the tubing by means of a rod. Brush the tubing with the flame of a Meker burner in order to drive off superficial moisture. Insert the tubing into the reaction tube, placing it firmly on the clay supports. Push the tubing and the clay supports to the center of the reaction tube by means of a stout iron wire bent at the end.

Table II. Hydrogen in Specially Prepared Hydrogen-Titanium Alloy Containing 0.97% Hydrogen[a]

	%
	0.94
	0.92
	0.92
	0.94
	0.93
	0.92
Av.	0.93

[a] Calculated from gain in weight of specimen.

Attach the copper oxide tube, place the Meker burner at full heat under it, and allow the oxygen to pass through for about 2 minutes. Attach the collection tube and the protective tower. Adjust the flow of oxygen to a rapid rate (about 400 ml. per minute), and place the furnace over the reaction tube. The sample will ignite in about 5 minutes. Fifteen minutes after the ignition, detach the collection tube, allow it to stand 5 minutes in the balance case, open momentarily to the atmosphere, and weigh. The gain in weight is water. Calculate the per cent hydrogen as follows:

$$\% \text{ hydrogen} = \frac{11.2\,(H - B)}{W}$$

where

H = weight of water (grams) obtained for run
B = weight of water (grams) obtained for blank
W = weight of sample (grams)

To prepare for the next run remove the furnace for the reaction tube and allow the reaction tube to cool while continuing the flow of oxygen at a slow rate. When the reaction tube has cooled to below 300° C., remove the 15-mm. 96% silica tubing and discard it. Weigh another sample and proceed as before.

To shut down the apparatus detach the copper oxide tube and store it in a desiccator. Attach to the reaction tube a protective tube containing Anhydrone held in place by glass wool. The protective tube is made of a piece of borosilicate glass tubing about 6 inches in length with a male 29/42 ground-glass joint at one end.

NOTES ON PROCEDURE

The ground-glass joints are essential. If rubber stoppers are used, high and erratic blanks will be obtained.

The temperature of the copper oxide in the purification tube must be about 750° C., and the length of the column at least 10 inches. If the temperature is much less than 700° C. or a short column is used, high blanks will result.

Glass wool should not be substituted for the crumpled copper wire to hold the copper oxide in place in the purification tube because glass wool may melt.

The reaction tube should be rotated 180° after every few runs to prevent sagging.

It is essential to have plugs of glass wool in the copper oxide tube in order to remove particles of lead oxide produced in the reaction.

The collection tube should be small to increase the accuracy of the weighings. The collection tube should not be allowed to become warm; otherwise erratic results will be obtained. The weight of the tube tends to become less when the tube becomes warm. The Ascarite and Anhydrone used in the purification tube or the collection tube should be tramped down with a rod to prevent the formation of an air space at the top.

The protective tower is necessary, as gas is drawn into the reaction tube from both ends of the system when the titanium starts to burn. A sulfuric acid trap cannot be substituted for the protective tower, because the sulfuric acid would be sucked back into the apparatus.

If necessary, the drillings should be pounded with a steel mortar and pestle to enable them to fit into the 15-mm. tubing.

It is essential that the sample be thoroughly cleaned with carbon tetrachloride to remove grease that is frequently present. The presence of grease will cause high results for hydrogen. The carbon tetrachloride must be completely evaporated, otherwise high results will be obtained for hydrogen. Ether, alcohol, and acetone are not satisfactory solvents for grease.

It is important that the 15-mm. tubing be brushed with a flame after the sample is put into it, in order to drive off any moisture that may be introduced in handling the tube. This treatment also drives off moisture from the surface of the titanium and the lead.

It is essential that the sample ignite and burn completely, otherwise very low results will be obtained for hydrogen. A fast flow rate of oxygen is necessary for complete burning.

The furnace must be heated to the operating temperature before it is put in place, as the proper ignition of the sample depends upon a high temperature being attained immediately.

When a sample of commercially pure titanium is ignited in the method, the melt is usually yellow, owing to the litharge (PbO) produced. When a sample of a titanium alloy is ignited, the melt is frequently brown or black. Occasionally some metallic lead is found at the bottom of the melt.

Experiments were carried out using many types of fluxes. Tin and iron were found to be ineffective. The use of various lead oxides caused erratic results because they contained organic material.

RESULTS

The results obtained for the proposed method on 11 standard samples, whose hydrogen content has been determined by vacuum fusion, are shown in Table I. The first four samples were obtained from Watertown Arsenal and the remaining samples from Wright Air Development Center. The results for samples from Wright Air Development Center are tentative and unofficial pending further compilation of data and a statistical study by that establishment. As can be seen from Table I, the accuracy and precision of the proposed method are satisfactory. The results obtained on a special hydrogen-titanium alloy obtained by heating titanium metal in a stream of high purity hydrogen at 500° C. are shown in Table II. As judged by gain in weight of the specimen, the hydrogen content of the sample was 0.97%. However, this result was probably somewhat high because of the absorption of small amounts of oxygen and nitrogen by the titanium.

LITERATURE CITED

(1) Alexander, P. P., *Metals & Alloys* **9**, 45 (1938).
(2) Anderson, K., and Fleshman, W. S., *Ind. Eng. Chem.* **42**, 1381 (1950).
(3) Anderson, K., and Grunewald, A. L., "Titanium Hydride," Fairchild Engine and Airplane Corp., NEPA Division, Oak Ridge, Tenn., 1948.
(4) Arkel, A. E. van, "Reine Metalle," p. 124, Julius Springer, Berlin, 1939.
(5) Armour Research Foundation, "Proceedings of the Symposium on Analysis and Metallography of Titanium," Chicago, Ill., June 1951, p. 26.
(6) Baker, T., *J. Iron Steel Inst., Carnegie Schol. Mems.* **3**, 249 (1911).
(7) Baker, W. A., Child, F. C., and Glaisher, W. H., *J. Inst. Metals* **70**, 373 (1944).
(8) Bardenheuer, P., and Ploum, H., *Mitt. Kaiser Wilhelm Inst. Eisenforsch., Düsseldorf* **16**, No. 11, 129 (1934).
(9) Bobalek, E. G., and Shrader, S. A., IND. ENG. CHEM., ANAL. ED. **17**, 544 (1945).
(10) Chrétien, P. E., Nipper, H. A., and Piwowarsky, E., *Aluminum-Arch.* **33** (No. 23) (1939); *J. Inst. Metals* **66** (Pt. 4), *Met. Abstracts* **7**, 146 (1940).
(11) Chufarov, G. I., and Nosyreva, S. S., *Zavodskaya Lab.* **13**, 287 (1947).
(12) Craighead, C. M., Lenning, G. A., and Jaffee, R. I., *J. Metals* **4**, 1317 (1952).
(13) Czochralzki, J., *Z. Metallkunde* **14**, 277 (1922).
(14) Derge, G., Peifer, W., and Alexander, B., *Trans. Am. Inst. Mining Met. Engrs.* **162**, 361 (1945).
(15) Dushman, S., "Scientific Foundations of Vacuum Technique," chap. 9, p. 547, Wiley, New York, 1949.
(16) Eastwood, L. W., "Gas in Light Alloys," pp. 15–19, Wiley, New York, 1946.
(17) Eastwood, L. W., and Kura, J. G., *Foundry* **75** (No. 7), 70 (1947).
(18) Gerke, F. K., *Zavodskaya Lab.* **13**, 298 (1947).
(19) Gerke, F. K., and Zolotareva, N. V., *Ibid.*, **4**, 19 (1935).
(20) Gibb, T. R. P., *J. Electrochem. Soc.* **93**, 198 (1948).
(21) Gibb, T. R. P., and Kruschwitz, H. W., *J. Am. Chem. Soc.* **72**, 5365 (1950).
(22) Gonser, B. W., *Ind. Eng. Chem.* **42**, 222 (1950).
(23) Goutal, E., *VIIth Int. Congr. Applied Chem., London* **1**, 50 (1909).
(24) Gulbransen, E. A., and Andrew, K. F., *J. Electrochem. Soc.* **96**, 364 (1949).
(25) Gulbransen, E. A., and Andrew, K. F., *Trans. Am. Inst. Mining Met. Engrs.* **185**, 741 (1949).
(26) Hartbower, C. E., and Daley, D. M., *Welding J. N. Y.* **33**, (No. 7), 311s (1954).
(27) Holm, V. C. F., and Thompson, J. G., *J. Research Natl. Bur. Standards* **26**, 245 (1941).
(28) Jaffee, R. I., and Campbell, I. E., *Trans. Am. Inst. Mining Met. Engrs.* **185**, 646 (1949).
(29) Lenning, G. A., Craighead, C. M., and Jaffee, R. I., *J. Metals* **4**, 1317 (1952).
(30) *Ibid.*, **6**, 367 (1954).
(31) McGeary, R. K., "Zirconium and Zirconium Alloys," Symposium, Am. Soc. for Metals, p. 168, Cleveland, Ohio, 1953.
(32) Mallett, M. W., *Trans. Am. Soc. Metals*, Preprint **41** (1948).
(33) Mallett, M. W., Gerds, A. F., and Griffith, C. B., ANAL. CHEM. **25**, 116 (1953).
(34) Metallurgical Advisory Committee on Titanium, Watertown Arsenal, "Status Bulletin on Unclassified Titanium Research and Development of Army Ordnance Corps," No. P12 (February 1954).
(35) Meyer, H. M., and Rostoker, W., *Welding J. N. Y.* **33**, 173s (1954).
(36) Moore, G. A., *Trans. Am. Inst. Mining Met. Engrs.* **162**, 404 (1945).
(37) Moreau, L., Chaudron, G., and Portevin, A., *Compt. rend.* **201**, 212 (1935).
(38) Muller, F. C. G., *Ber.* **12**, 93 (1879).
(39) National Research Corp., Cambridge, Mass., "Operation Instructions for Vacuum Fusion Analysis," 1952.
(40) Naughton, J., *Trans. Am. Inst. Mining Met. Engrs.* **162**, 385 (1945).
(41) Pearson, T. F., Baker, W. A., and Child, F. C., *Proc. Inst. Brit. Foundrymen*, Paper **904**, 1948.
(42) Pepkowitz, L. P., and Proud, E. R., ANAL. CHEM. **21**, 1000 (1949).

(43) Portevin, A., Chaudron, G., and Moreau, L., *Compt. rend.* **204**, 1252 (1937).
(44) Post, C. B., and Schoffstall, D. G., *Trans. Am. Inst. Mining Met. Engrs.* **162**, 390 (1945).
(45) Rooney, T. E., and Barr, G., *J. Iron Steel Inst. London* **119**, 575 (1929).
(46) Rummel, T., Esmarch, W., and Beuther, K., *Metallwirtschaft* **19**, 1029 (1940).
(47) Scafe, R. M., *Trans. Am. Inst. Mining Met. Engrs.* **162**, 375 (1945).
(48) Schmid, E., and Schweinitz, H. D. von, *Aluminium* **21**, 772 (1939).
(49) Schwartz, H. A., and Guiler, G. M., *Trans. Am. Foundrymen's Assoc.* **47**, 742 (1939–40).
(50) Scott, W. W., "Standard Methods of Chemical Analysis," vol. I, p. 443, Van Nostrand, New York, 1939.
(51) Shields, B. M., Chipman, J., Grant, N. J., and Carney, D. J., *Materials & Methods* **40** (No. 1), 220 (1954).
(52) Sloman, H. A., Harvy, C. A., and Kubaschewski, O., *J. Inst. Metals* **80**, 391 (1951–2).
(53) Smith, D. P., "Hydrogen in Metals," p. 160, University of Chicago Press, Chicago, 1948.
(54) Smithells, C. J., "Metals Reference Book," p. 380, Interscience, New York, 1949.
(55) Sterner-Rainer, R., *Z. Metallkunde* **23**, 274 (1932).
(56) Swain, A. J., *J. Inst. Metals* **75**, 863 (1949).
(57) Tammann, G., and Schneider, J., *Z. anorg. u. allgem. Chem.* **172**, 43 (1928).
(58) Thompson, J. G., *Trans. Am. Inst. Mining Met. Engrs.* **162**, 369 (1945).
(59) Titanium Metals Corp. of America, New York, "Handbook on Titanium Metal," pp. 66, 75, 89, 1952.
(60) Veinberg, G. Ya., and Proshutinskii, S. I., *Zavodskaya Lab.* **6**, 422 (1937).
(61) Walter, D. I., ANAL. CHEM. **22**, 297 (1950).
(62) Wells, J. E., and Barraclough, K. C., *J. Iron Steel Inst. London* **155**, 27 (1947).
(63) Willems, F., *Aluminium* **23**, 337 (1941).
(64) Yao, Y. L., and Milliken, K. S., ANAL. CHEM. **25**, 363 (1953).
(65) Yavoiskii, V. I., *Zavodskaya Lab.* **11**, 406 (1945).
(66) *Ibid.*, **13**, 262 (1947).
(67) Yeaton, R. A., *Vacuum* **2**, 115 (1952).
(68) Yenson, T. D., and McGeary, R. K., *Trans. Am. Inst. Mining Met. Engrs.* **162**, 355 (1945).

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Liquid Scintillation Counter for Carbon-14 Employing Automatic Sample Alternation

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A scintillation counter for carbon-14, which uses solution phosphors, has been described. This instrument has been used to measure the activity ratio of a pair of carbon-14-containing solutions with an average deviation of approximately 0.5% from the mean and from the calculated value. It employs automatic alternate counting of three cells, one of which is usually a standard and one a background. The alternating feature was designed to reduce possible effects of instrument drift and changing background on the results from long counting periods. A series of experiments to test this feature is reported. The counter is simple, electronically. The mechanical parts are not difficult nor expensive to build where adequate shop facilities are available.

THE vibrating reed electrometer when used with a Borkowski-type ionization chamber is a standard instrument for the assay of carbon-14 compounds. The organic compound is burned to form carbon-14 dioxide, which, when introduced into the ionization chamber, produces a current which is measured by the electrometer (*5*, *6*). Scintillation counting (*2*, *3*) of carbon-14 compounds dissolved in organic solvents containing scintillators such as terphenyl has the advantage that the compound need not be burned. Several instruments have been described for this type counting.

The present paper discusses the design and testing of one such instrument. The principal objective was to obtain an instrument which would measure precisely and reproducibly the ratio of activities contained in two or more samples of the same compound, without regard for whether or not the individual measurements of the activity of any sample varied from time to time. It is well known that for most tracer studies ratios of activities of several samples are needed, whereas the absolute activity of any one sample is not needed. For this reason an automatic sample changer was designed to allow three samples (one to measure the background which could be subtracted from each of the other two before calculation of their ratio) to be counted alternately for short intervals with the integrated counts for each sample stored on a separate register. It was felt that such an alternating system could reduce the effect of instrument drift and probably permit ratios of activities of several samples to be measured with greater precision than that obtained with instruments not employing sample alternation.

The use of refrigerated systems employing two photomultipliers, two amplifiers, and a coincidence circuit to reduce background has been described by several writers (*1*, *4*), and a commercially available unit has been recently introduced (*7*). These dual channel systems are characterized by high efficiencies for carbon-14 (values as high as 75% have been reported) and by low backgrounds (100 to 200 counts per minute). However, they are bulky, since refrigerators and rack mounting are required, and the electronic circuitry is fairly complex. These systems also require that the counting cell and contents be refrigerated to 0° C. or below, which in general decreases the solubility of the scintillator and the sample in the solvent. Finally, they are expensive. For the present purpose it seemed that a counter using one refrigerated phototube and one amplifier would be more stable, easier to maintain, and, incidentally, less expensive than one using two such units and a coincidence circuit plus a large refrigerator. The amplifier and scaling unit employed in the counter described here are those of a commonly used proportional counter; thus it may appeal to those who already possess such equipment.

DESIGN

Detector. The detector is a 1P21 photomultiplier which is operated at liquid nitrogen temperature. The tube rests with its base up on the bottom of a 500-ml. Dewar flask with its cathode facing a 1.25 × 2 inch unsilvered window in the side of the flask. The base of the phototube is partially removed by chucking the base end in a lathe and cutting away the upper part of the base until there is no mechanical connection with the glass envelope, except that of the wire leads. This is done in order to eliminate the strains on the envelope and on the soldered connections to the prongs of the base, which are caused by the drastic cooling which occurs when liquid nitrogen is poured over the phototube.

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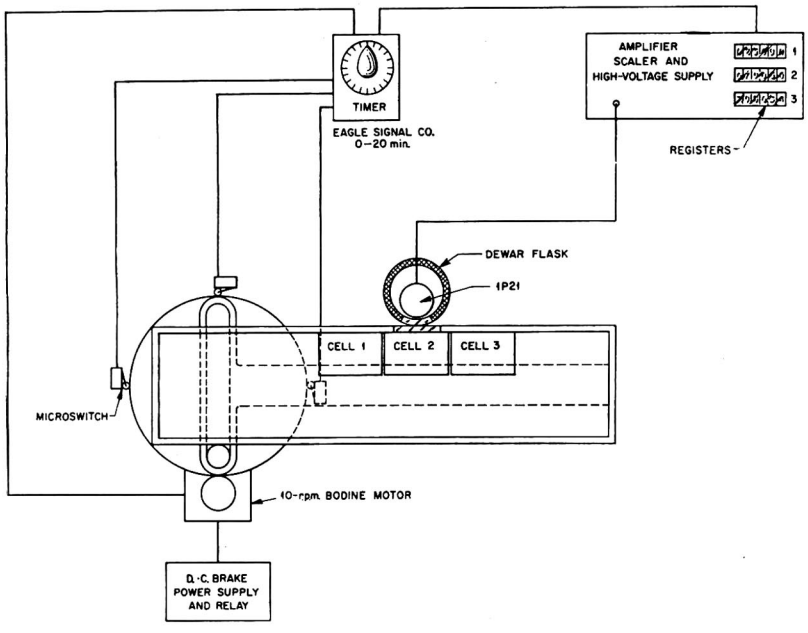

Figure 1. Schematic diagram of counting system

Although part of the base of the tube is removed, it remains rigid and is easily plugged into a standard socket around which are wired the voltage-divider resistors. During operation these resistors are also at liquid-nitrogen temperature. The high voltage and signal leads to the phototube, which serve to keep the phototube in place in the Dewar flask, are brought out to connectors mounted on the side of the sheet brass housing. The phototube output is developed across a 15,000-ohm load resistor and is fed directly to the input of the amplifier through an 8-inch coaxial cable.

Sample Changer. An alternating sample changer was included which would count, in short cycles, the cell series: sample, background, and standard. Figure 1 is a schematic diagram of the system. It consists of a carriage operated by a yoke driven by a Bodine motor at 10 r.p.m. Inside the carriage housing (Figure 2, top view) are located 3 double pole, double throw micro switches which stop the motion of the carriage, position the cell being counted in front of the window, switch in the appropriate register, and start the timer. At the end of the timing period, which may be from 1 to 20 minutes, the carriage moves the next sample in place and the timing cycle is repeated. In order to position the cells accurately and to prevent the carriage from coasting through a position without counting, a direct current braking current is applied to the windings of the motor driving the carriage. The positioning of each sample in front of the window is reproducible to within approximately ±0.5 mm. By means of a switch either two or three samples may be included in the counting cycle.

The cells used are standard rectangular spectrophotometer cells of approximately 19-ml. capacity. They are contained in removable holders which are held in position on the carriage by dowel pins (see Figure 2). On the back of the holder is an aluminum reflector. The cell is held in the cell holder by means of a spring loaded yoke, which also keeps the lid firmly on the cell and makes it light tight, except for the opening seen by the phototube. Glass plate lids were used. The dimensions of the window in the cell holder are several millimeters larger than those of the opening seen by the phototube, so that the geometry is essentially constant. The changer may be manually operated by setting the timer for a short interval, say 5 seconds, and bringing the desired sample into position. The timer is then turned off.

Table I. Measured Ratios of Successive Daily Pairs of Samples Having a Calculated Ratio of 2.000

(Samples were counted alternately for the interval shown. Total counting time for a given interval was 4 hours)

Interval Time, Minutes	Sample Pair Number 1	2	3	4	5	6	Av. for Interval	% Dev.[a] from Calcd. Ratio
1	1.993						1.993	−0.4
5	1.981	1.973					1.977	−1.1
10		1.980	1.975				1.978	−1.0
20			1.970				1.970	−1.5
60				2.002	1.952	1.970	1.975	−1.3
120					1.934	1.996	1.965	−1.8

[a] All negative, because the count rate for each sample decreased with time and each run was started with the lower activity sample in place before the phototube. The counting rate decreased for approximately 3 hours. This was undoubtedly due to decaying phosphorescence.

Table II. Results Obtained from Counting Same Pair of Samples Over Period of 32 Hours for 1-Minute, 1-Hour, and 2-Hour Intervals

(Calculated ratio = 2.000)

4-Hour Period from Which Ratio Was Taken	Ratio of Counts: 10 one-minute intervals	2 one-hour intervals	2 two-hour intervals
1	1.983	1.970	1.947
2	1.989	1.997	1.996
3	1.990	1.989	1.996
4	2.015	1.995	1.998
5	1.996	1.989	1.980
6	2.001	1.966	1.962
7	2.003		
Mean	1.997	1.984	1.980
Av. dev. of mean	±0.008	±0.011	±0.017
% dev. of mean from calcd. value	−0.15	−0.8	−1.0

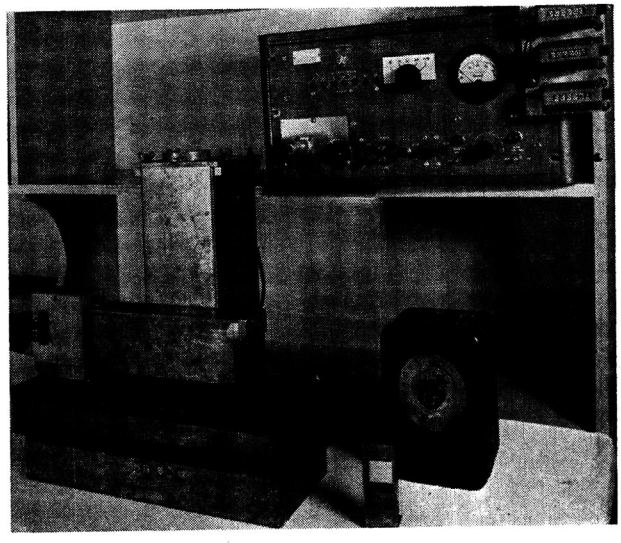

Figure 2. Assembled counting system

Amplifier and Scaler. The counter proper is a Nuclear Instrument and Chemical Co. Model 162 proportional counter, which had been modified by the Chemistry Division Instrument Group for improved signal to noise ratio and dynamic range when used with a beta proportional counter. The modified circuit was retained except for the diode load at the input. This was replaced by a 15,000-ohm resistor. The high voltage supply was rebuilt according to Drawing Q 1144-1 of Oak Ridge National Laboratory. This circuit was changed slightly to give 700 to 1400 volts and was connected as a positive supply. It possesses excellent regulation and is free from the tube selection which was necessary in the original Model 162 supply. The 0 to 1 ma. meter in the counter was replaced with a 0 to 200 μa. meter, and provision was made for monitoring the phototube voltage with a potentiometer. [Circuit diagrams may be obtained from one of the authors (J. B. D.).] A switch operated by the sliding door of the cell

Table III. Sample of Data for Sample Pairs 1 and 5 from Table I

Sample Pair	Alternation Interval, Minutes	Total Running Time, Hours	Accumulated Register Counts[a] Gross A	Gross B	Background	Net A	Net B	Ratio B/A	Net Counting Rate Register Counts/Min. Start of run A	Start of run B	End of run A	End of run B
1	1	4	6,113	12,054	128	5,985	11,926	1.993	91	184	84	170
5	120	4	10,234	19,645	140[b]	10,094	19,505	1.934	84	165	81	160

Data from 32-Hour Run Showing Drop in Counting Rate

Time after Start	Counting Rate Register Counts/Min. A	B
1 min.	92	182
10 min.	88	177
4 hr.	83.3	165.6
16 hr.	85.5	170.7
32 hr.	81.6	163.5

[a] Register counts × 128 = actual counts.
[b] Estimated from short counts at beginning and end of 2-hour run.

housing removes the voltage from the phototube when the door is opened. The scaler section of the counter was unchanged except for the mounting of the three registers on the front panel and the inclusion of a scaling factor switch. The scaling factor switch allows the selection of the factor which stores the maximum number of counts on the registers without exceeding their speed limitations. (Figure 2 shows the assembled system.)

EXPERIMENTAL

Preparation of Solutions for Test Runs. A stock solution (I) of 8.5 grams of recrystallized terphenyl in 2 liters of C. P. xylene was prepared. Each cell used for counting background contained 15 ml. of this solution. Stock solution II was obtained by dissolving 20 mg. (20 μc.) of benzoic-α-carbon-14 acid in 1 liter of stock solution I. Stock solution III was obtained by mixing equal volumes of solution I and solution II. Thus, all three stock solutions contained the same concentration of terphenyl, but different concentrations of labeled benzoic acid. Fifteen milliliters of solution II (0.30 μc.) were pipetted into a clean cell, and 15 ml. of solution III (0.15 μc.) were pipetted into a second clean cell in order to obtain a pair of cells with 2 to 1 ratio of activities. After each run the solutions were discarded and the cells and their plate glass tops were washed thoroughly with xylene followed by acetone. They were then dried. No difference within experimental error was found when the cells, holders, and sample positions were interchanged.

RESULTS AND DISCUSSION

Tables I and II show the precision obtained when the ratio of the activity above background of two benzoic acid samples was determined. Table III gives a sample of the data from which the ratios in Tables I and II were calculated. The shorter counting intervals gave ratios which deviated somewhat less from the calculated value and from the mean. The superiority of short time intervals is a rough measure of the value of the alternating feature of the instrument. The counting efficiency for carbon-14 for the results in the tables was approximately 3%.

LITERATURE CITED

(1) Arnold, J., *Science* **119**, 155 (1954).
(2) Farmer, E. C., and Berstein, J. A., *Ibid.*, **115**, 460 (1952).
(3) Hayes, F. N., Hiebert, R. D., and Schuch, R. L., *Ibid.*, **116**, 140 (1952).
(4) Hiebert, R. D., and Watts, R. J., *Nucleonics* **11**, 38 (1953).
(5) Neville, O. K., *J. Am. Chem. Soc.* **70**, 3501 (1948).
(6) Raaen, V. F., and Ropp, G. A., ANAL. CHEM. **25**, 174 (1953).
(7) Tracerlab, Inc., Boston, Mass., Tracerlog, No. 59 (April 1954).

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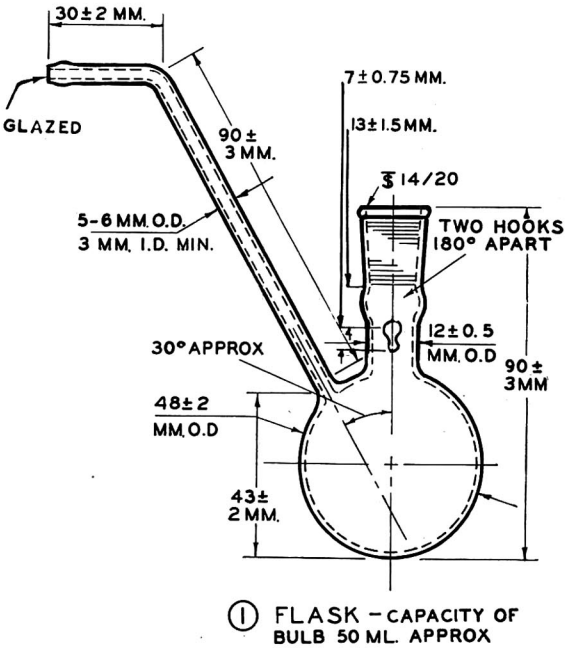
Report on Recommended Specifications for Microchemical Apparatus

Alkoxyl

IN PREVIOUS reports (*1, 9, 13–17*) of the Committee on Microchemical Apparatus, recommended specifications were published for pieces of apparatus which were either the most widely used for the work in question, or shown to be an improvement over the more widely used apparatus through tests made by the members of the committee and other cooperating chemists. In this report specifications are suggested for the semimicro alkoxyl apparatus, which has been selected on the basis of being the most widely used.

Recommended specifications for an apparatus for the determination of alkoxyl groups were delayed until a collaborative study (*10, 11, 18*) of methods for this determination had been conducted by the Association of Official Agricultural Chemists. In this study, compounds representing ethyl and methyl esters and ethers were submitted to practicing microchemists, who had expressed their willingness to cooperate. These individuals were asked to analyze the samples by whatever methods they were using in their own laboratories and to furnish detailed information on the procedure and apparatus. Where enough collaborators used a particular procedure or apparatus to permit the results to be treated statistically, calculations were made to determine which of these or their variations appeared to give the best accuracy and/or precision.



Report Prepared by

Committee on Microchemical Apparatus, Division of Analytical Chemistry, AMERICAN CHEMICAL SOCIETY

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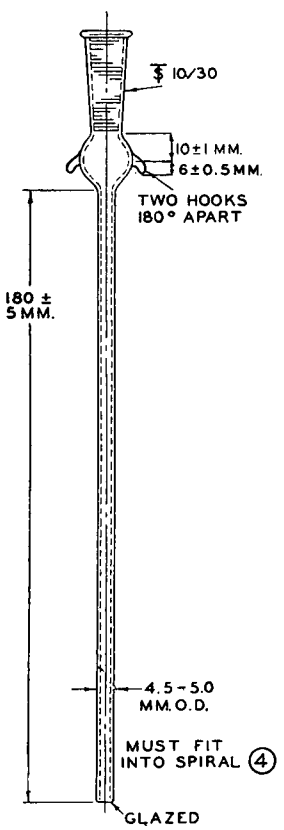
(See page 114 for ②)



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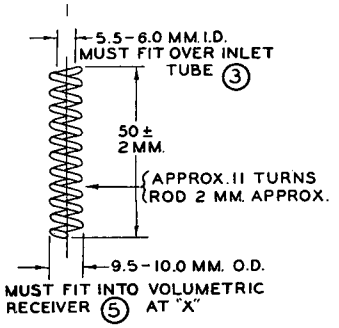

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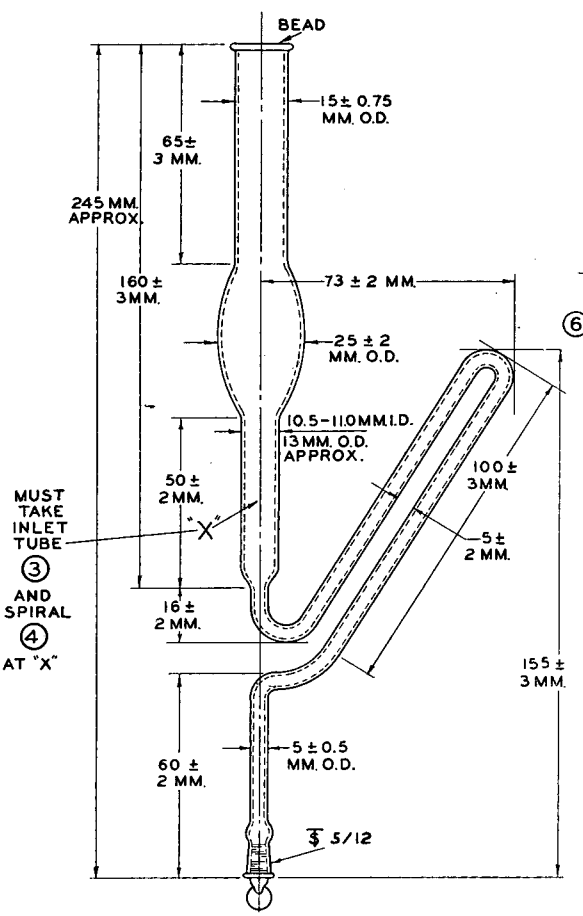
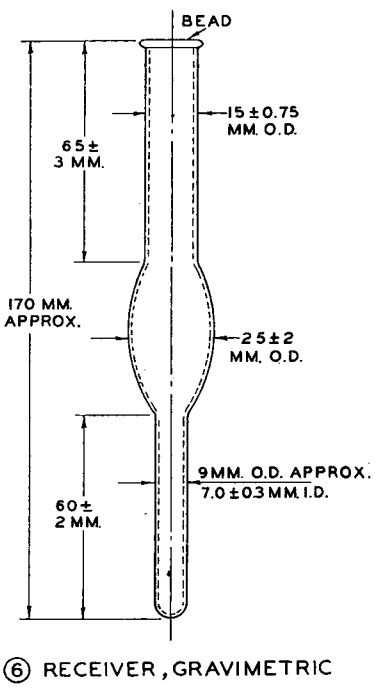


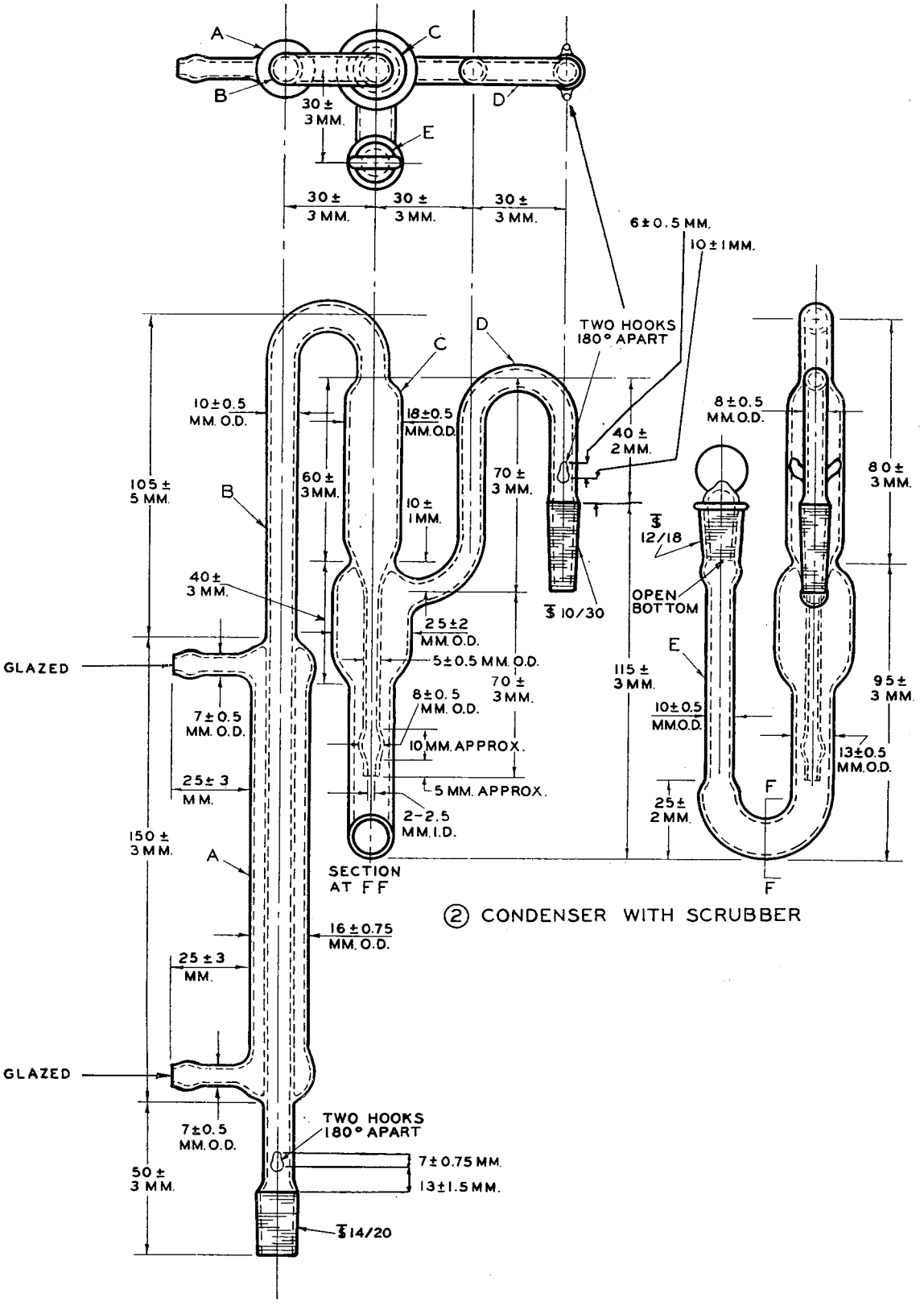
⑤ RECEIVER, VOLUMETRIC



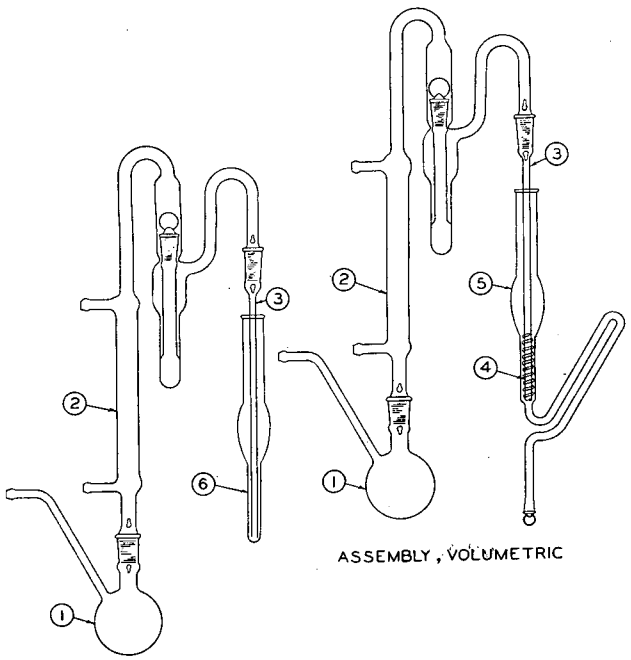
⑥ RECEIVER, GRAVIMETRIC

In general, the Clark apparatus (*2–5*) has been considered to be suitable for semimicro work, or where relatively large amounts of material must be taken in order to analyze for small percentages of alkoxyl groups. The results of the above studies indicated that the Clark apparatus is widely used and that it represents a model with which a large variety of compounds can be successfully analyzed on the micro, as well as the semimicro, scale.

In the studies made by the Association of Official Agri-



② CONDENSER WITH SCRUBBER



cultural Chemists, only the volumetric procedure was used. It is well known, however, that reliable results can be obtained gravimetrically (*6–8, 12*). Therefore, the Committee on Microchemical Apparatus recommends specifications for a Clark-type apparatus, illustrated in the figures, which can be used for either procedure. For the volumetric procedure, the apparatus consists of the reaction flask with side arm ①, condenser with scrubber ②, inlet tube ③, spiral ④, and volumetric receiver ⑤ and is shown assembled. For the gravimetric procedure, the spiral ④ and volumetric receiver ⑤ are replaced by gravimetric receiver ⑥, and this is also shown assembled.

The dimensions for the side arm of the flask ① were arrived at after a number of experiments. Capillary tubes, with and without bulbs, were unsatisfactory because of condensation in the tube. The recommended length of the side arm is necessary to minimize contact of acid with the gas connection.

The condenser with scrubber ② has an enlarged section between the two parts to prevent suck-back of liquid from scrubber into condenser at the end of a determination. Several types of scrubbers were tested, including one constructed of two compartments connected by a capillary tube. The one selected operated more efficiently than all others tried.

The section between the scrubber and the inlet tube ③ was designed to prevent liquid being carried into the receiver.

Use of the spiral ④ in the receiver ⑤ is optional in the volumetric procedure. Extensive tests have shown that equally good results are obtained without the spiral.

This apparatus was used in the collaborative study conducted by the Association of Official Agricultural Chemists this year (*11*), and good results were obtained by the 13-collaborating microanalysts who reported a total of 198 determinations on four samples [benzocaine (ethyl *p*-aminobenzoate), *p*-ethoxybenzoic acid, methyl *p*-aminobenzoate, and vanillin (4-hydroxy-3-methoxybenzaldehyde)].

Alkoxyl apparatus of smaller dimensions than the one recommended has been described for the microdetermination of alkoxyl groups (*6–8, 12*). The committee has considered these, but believes that further investigation is needed before any recommendations can be made.

LITERATURE CITED

(1) ANAL. CHEM. **21**, 651 (1949).
(2) Association of Official Agricultural Chemists, Washington, D. C., "Official Methods of Analysis of Association of Official Agricultural Chemists," 7th ed., p. 744, 1950.
(3) Clark, E. P., *J. Assoc. Offic. Agr. Chemists* **15**, 136 (1932).
(4) *Ibid.*, **22**, 622 (1939).
(5) Clark, E. P., "Semimicro Quantitative Organic Analysis," p. 68, Academic Press, New York, 1943.
(6) Grant, J., "Quantitative Organic Microanalysis, Based on the Methods of Fritz Pregl," 5th English ed., p. 182, Blakiston, Philadelphia, 1951.
(7) Roth, H., "F. Pregl Quantitative Organische Mikroanalyse," 5th ed., p. 215, Springer-Verlag, Vienna, 1947.
(8) Steyermark, Al, ANAL. CHEM. **20**, 368 (1948).
(9) *Ibid.*, **22**, 1228 (1950).
(10) Steyermark, Al, *J. Assoc. Offic. Agr. Chemists* **38**, 367 (1955).
(11) *Ibid.*, in press (probable publication date, May 1956).
(12) Steyermark, Al, "Quantitative Organic Microanalysis," p. 230, Blakiston, Philadelphia, 1951.
(13) Steyermark, Al, Alber, H. K., Aluise, V. A., Huffman, E. W. D., Jolley, E. L., Kuck, J. A., Moran, J. J., and Willits, C. O., ANAL. CHEM. **23**, 1689 (1951).
(14) Steyermark, Al, Alber, H. K., Aluise, V. A., Huffman, E. W. D., Jolley, E. L., Kuck, J. A., Moran, J. J., Ogg, C. L., and Willits, C. O., *Ibid.*, **26**, 1186 (1954).
(15) Steyermark, Al, Alber, H. K., Aluise, V. A., Huffman, E. W. D., Kuck, J. A., Moran, J. J., and Willits, C. O., *Ibid.*, **21**, 1283 (1949).
(16) *Ibid.*, p. 1555.
(17) *Ibid.*, **23**, 523 (1951).
(18) Steyermark, Al, and Loeschauer, E. E., *J. Assoc. Offic. Agr. Chemists* **37**, 433–49 (1954).

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X-Ray Diffraction Patterns of Phenols—Correction

In the article on "X-Ray Diffraction Patterns of Phenols" [Hofer, L. J. E., and Peebles, W. C., ANAL. CHEM. **27**, 1852 (1955)] on page 1856 in Table II the heading of the second column for 2,4-Dimethyl-6-isobornylphenol should be I/I_1.

Effect of pH on High-Salt–Thorium Fluoride Titration

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Variations in pH of the final titration solution are shown to increase the variability with which microgram quantities of fluoride may be determined. A graphic correction of the observed titration volume based upon the pH of the final titration solution increases the precision of the method. The effect of small changes in pH of the final titration solution is also reduced by conducting the titration at 2.90 instead of at 2.70 as previously recommended.

THE salt-acid–thorium fluoride titration method of Williams (*8*) as modified by Smith and Gardner (*4–6*) offers several advantages over the previously used thorium-alizarin titration (*1*) of microgram quantities of fluorides. The primary advantages of the modified titration procedure are the use of a single titration against a permanent color standard and the control of interfering ions and dissociation by the introduction of sodium chloride. The modified procedure was found to be more satisfactory than the back-titration procedure for routine fluoride analysis. However, it was observed upon utilization of this method over a period of time that the titration was not always as reproducible as might be anticipated.

A study of the initial and final pH of titrated replicated samples indicated that the apparent fluoride content, as measured by the volume of thorium reagent required, was an inverse function of the pH of the final titration volume. The proposed modification is based on a study of the effect of the pH of the final titration values upon the observed quantity of fluoride.

REAGENTS

Acidified thorium nitrate solution. Dissolve 0.268 gram of thorium nitrate tetrahydrate in distilled water, dilute to nearly 1 liter, adjust the pH to 2.90 with 1.0*N* hydrochloric acid using a pH meter, and complete dilution to 1 liter.

Acid-indicator solution. Dissolve 0.020 gram of monosodium alizarin sulfonate (Alizarin Red S) in water, add 16.1 ml. of 1.0*N* hydrochloric acid, and dilute to 200 ml.

Salt solution, 5*N* sodium chloride.

Hydroxylamine hydrochloride solution, 1%.

Hydrochloric acid solutions, 1.0 and 0.1*N*.

Sodium hydroxide solution, 0.1*N*.

Permanent color standard. Prepare a stock solution containing 6.0 ml. of 0.65*N* hydrochloric acid, 52.0 ml. of 3.66% cobaltous chloride ($CoCl_2.6H_2O$), and 4.0 ml. of 0.10% potassium chromate, and dilute to 100 ml. Dilute 5.0 ml. of this stock solution to 100 ml. with distilled water in a Nessler tube. Keep the Nessler tube containing the permanent color standard tightly stoppered when not in use.

Sodium fluoride solution. Dry reagent grade sodium fluoride in an oven at 110° C. for 24 hours. Cool in a desiccator, weigh 0.221 gram, dissolve, and dilute to 1 liter with distilled water. Dilute 100 ml. of the stock solution to 1 liter to obtain a solution containing 10 γ of fluorine per milliliter. Store both stock and dilute fluoride solutions in wax-lined or polyethylene bottles.

PROCEDURE

The following procedure is used for determining 1 to 100 γ of fluoride in solutions containing sodium hydroxide.

Isolate the fluorides from the original samples by the usual procedure of either single or double distillation (*1–3, 7*), the distillate being neutralized with sodium hydroxide. Transfer an aliquot of the distillate of not more than 80 ml. into a 150-ml. beaker. If a smaller aliquot than 80 ml. is required to obtain a fluoride concentration below 100 γ, add sufficient distilled water to the aliquot to make the aliquot volume up to 80 ml. Add 1.0 ml. of the hydroxylamine hydrochloride to reduce any chlorine that might be present in the distilled sample (*1*), 5.0 ml. of the sodium chloride solution to control dissociation and suppress interference (*8*), and 2.0 ml. of the acidified alizarin indicator. Adjust the pH of the resultant solution to 2.90 with 0.1*N* hydrochloric acid using a pH meter. Use 0.1*N* sodium hydroxide, if necessary, in making the adjustment to 2.90. Transfer the pH-adjusted sample to a 100-ml. Nessler tube. Titrate the solution with acidified thorium reagent against a daylight fluorescent background using a 10-ml. microburet. Continue titration by the addition of small increments of thorium reagent until the color of the indicator matches the color of the permanent color standard. Standardize each new thorium reagent against the standard 10 γ per ml. fluoride solution covering the titration range of 1 to 100 γ of fluoride. Plot the amount of thorium required in milliliters against the micrograms of fluoride. A straight line may be drawn for the fluoride range up to about 50 γ of fluoride. A formula for the calculation of the total micrograms of fluoride found in the sample may be used for the straight-line portion of the curve:

(Ml. of thorium for sample − ml. of thorium for blank) × (thorium titer, γ fluoride/ml.) × (total vol. of sample)/(vol. of aliquot titrated) = γ of fluoride

Calculations above the straight-line portion of the curve must be made by reference to the titration curve.

EXPERIMENTAL

Five replicated samples of fluoride at five different concentration levels—0, 20, 40, 60, and 80 γ—were adjusted to an initial pH of 2.6. Similarly, replicated samples at each of the five concentrations were adjusted to an initial pH of 2.7, 2.8, 2.9, 3.0, and 3.1. These samples were titrated as outlined in the procedure. Following completion of each titration, the pH of the final titration volume was determined. The volume of thorium reagent used was plotted against the final observed pH. As evidenced by the family of curves in Figure 1, the apparent fluoride concentration as indicated by the volume of thorium used is inversely related to the pH of the final titration volume. Furthermore, as the pH becomes more acid, the titration system becomes more sensitive to small changes in pH. The sensitivity of the system for fluoride decreases as the pH becomes more alkaline.

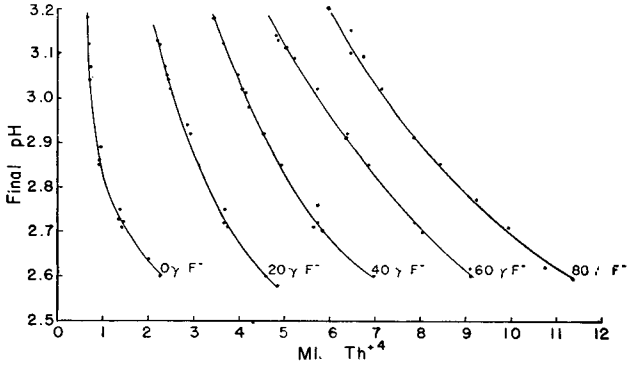

Figure 1. Effect of pH on volume of standard thorium required in high-salt–sodium fluoride titration

It is therefore proposed that the titration be carried out at an initial pH of 2.90 rather than the 2.70 as outlined by Smith and Gardner. This change represents a compromise between the minimum fluoride titer of the thorium reagent and the maximum changes in apparent fluoride concentration with small variations in pH of the final titration volume.

Forty samples in the concentration range of 20 to 80 γ of fluoride and pH range of 2.9 were titrated according to the analytical procedure outlined above. The fluoride content of each sample was then calculated in two manners: The actual volume of thorium nitrate required to titrate each sample was used, regardless of the pH of the final titration volume; and the volume of thorium nitrate was corrected for variation in pH of the final titration volume from the recommended 2.90 and used as the basis for calculation.

Table I summarizes the comparative results obtained by the two methods of calculation. The mean per cent recoveries obtained by the two calculation procedures show excellent agreement. However, the range when no correction is made for variations in pH of the final titration volume is somewhat greater than that obtained when the suggested correction for pH variation is made. Comparison of the standard deviations obtained by the two calculation procedures indicates a marked increase in precision by correcting the volume of thorium nitrate used to a standard pH according to the proposed procedure.

Table I. Determination of Known Quantities of Sodium Fluoride

	Uncorrected for pH	Corrected for pH
Number of samples	40	40
Concentration range, γ	20–80	20–80
Mean recovery, %	100.4	99.7
Range of recovery, %	92.5–107.8	93.8–103.7
Standard deviation, %	7.3	2.8
Range of pH	2.83–2.94	2.83–2.94

CORRECTION OF APPARENT FLUORIDE LEVEL

In order to improve the reproducibility of the modified Williams titration on replicated samples, the titration volume of thorium reagent required for a given sample must be corrected for the effect of variations on pH from 2.90 of the final titration solution. A graphic correction may be made by reference to a family of curves such as shown in Figure 1. The point of intersection of the final pH and the observed volume of thorium is first located within this family of curves, and the point is then moved in a manner parallel with the family of curves, either upward or downward, until it intersects the ordinate representing a pH of 2.90. The actual volume of thorium nitrate which would have been required by the given quantity of fluoride at a pH of 2.90 is then determined at the vertical intersection of the point at 2.90 and the abscissa.

LITERATURE CITED

(1) Assoc. Offic. Agr. Chemists, Washington, D. C., "Official Methods of Analysis," 7th ed., 1950.
(2) Remmert, L. F., Parks, T. D., Lawrence, A. M., and McBurney, E. H., ANAL. CHEM. 25, 450 (1953).
(3) Rowley, R. J., Grier, J. G., and Parsons, R. L., *Ibid.*, 25, 1061 (1953).
(4) Smith, F. A., and Gardner, D. E., *Arch. Biochem.* 29, 311 (1950).
(5) Smith, F. A., and Gardner, D. E., *J. Dental Research* 30, 182 (1951).
(6) Smith, F. A., and Gardner, D. E., U. S. Atomic Energy Commission, Bull. **AECD-2161**, 1948.
(7) Willard, H. H., and Winter, O. B., IND. ENG. CHEM., ANAL. ED. 5, 7 (1933).
(8) Williams, H. A., *Analyst* 71, 175 (1946).

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Velocity Barrier to Eliminate Absorption of Carbon Dioxide during Titrimetric Procedures

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Absorption of acidic and basic gases from laboratory air is a difficulty associated with titrations involving solutions of low buffer capacity. Methods of enclosing the solution in an inert atmosphere are generally cumbersome and not adaptable to the routine treatment of many samples. A simple and convenient new method permits access to the titration vessel at all times. A flat stream of nitrogen is directed horizontally across the top of the titration vessel so as to form a seal of rapidly moving nitrogen through which the acidic and basic gases may not diffuse. This velocity barrier is useful in the assay of ribonuclease activity.

DURING the development of a micromethod for the quantitative determination of ribonuclease based upon the acidic groups which are liberated from the substrate (ribonucleic acid), it became necessary to carry out titrations on a small scale (2 ml.) on solutions of low buffer capacity (0.3μ eq. per pH unit), with the assurance that no absorption of acidic or basic gases from laboratory air would occur.

Several arrangements for the use of nitrogen were tried. The most efficient and convenient arrangement was found to be a nozzle constructed from Lucite, as shown in Figure 1. This nozzle fixes the position of the titration vessel with respect to the stream of nitrogen. The stream of nitrogen functions in

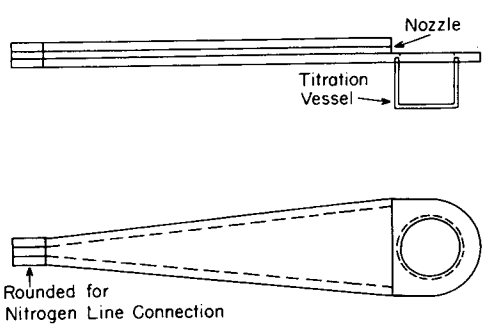

Figure 1. Lucite nitrogen nozzle

two ways to prevent the absorption of laboratory gases: (1) An aspirator-type action removes the gases from between the liquid and the streaming nitrogen, replacing them with nitrogen and water vapor from the sample; and (2) a velocity barrier action decreases the probability that a molecule of carbon dioxide, for example, could diffuse through the rapidly moving stream and into the titration vessel. The efficiency of the first action depends upon the velocity of the stream and its orientation with respect to the titration vessel. For this reason the titration vessel is shown to have a fixed position with respect to the efferent

nitrogen (Figure 1). The second action depends upon the thickness and velocity of the moving stream. A flow rate of 7 to 8 cubic feet per hour has been found to be sufficient.

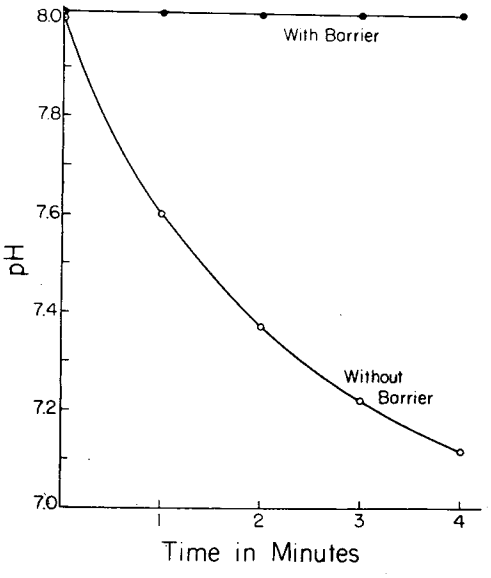

Figure 2. Action of velocity barrier

Figure 2 demonstrates the action of the velocity barrier. If 2 ml. of distilled water are adjusted to pH 8 by the use of 0.01*N* sodium hydroxide and stirred with no precautions against the uptake of carbon dioxide from the air, a rapid decrease in pH can be shown to occur. However, when the nitrogen barrier is placed in the system the pH remains constant.

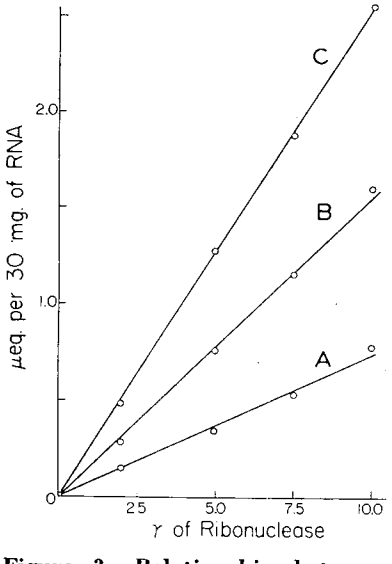

Figure 3. Relationship between titer and quantity of ribonuclease

A. Titers between 4 and 16 minutes
B. Interval of 4 to 34 minutes
C. Interval of 4 to 60 minutes

The merits of this type of velocity barrier under typical experimental conditions are illustrated in the following analytical procedures for the assay of ribonuclease action.

Thirty milligrams of ribonucleic acid are dissolved in 2 ml. of 0.25*N* sodium sulfate, for optimal ionic strength, and the solution is adjusted to pH 7.50 by means of a micrometer syringe buret which delivers 0.01*N* sodium hydroxide. The pH is measured on a Beckman Model G pH meter with shielded external electrodes. The solution is stirred magnetically and absorption of carbon dioxide is prevented by means of the nitrogen barrier applied across the top of the vessel.

Ribonuclease (2 to 10 γ) is added to the adjusted nucleic acid solution under the nitrogen barrier and the pH is brought to 7.50 and maintained by continuous addition of 0.01*N* sodium hydroxide from the syringe buret. After the first 4 minutes of reaction, during which there is no reproducible relationship between acidic groups formed and the amount of enzyme added, the titer for any interval up to an hour is directly proportional to the amount of enzyme present, as shown in Figure 3. Errors due to the setting precision of the meter may be reduced by plotting the titer every 2 minutes and drawing the best straight line through the data for a period of 10 to 15 minutes. A total time of 20 minutes per sample is adequate and after the titration vessel has been emptied by aspiration and washing, the next sample may be started in the same vessel.

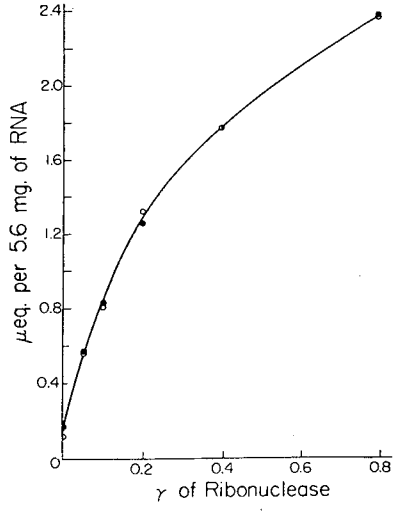

Figure 4. Relationship between titer, after 24 hours at 50° C., and quantity of ribonuclease

Open circles represent one series of hydrolyses and closed circles are for second series started immediately after first series

In order to conserve ribonucleic acid and to increase the assay sensitivity the enzymatic reaction may be carried out with 5 mg. of nucleic acid and 0.05 to 0.8 γ of ribonuclease in 2 ml. of 0.25*N* sodium sulfate incubated at 50° for 24 hours by transferring a portion of the adjusted solution to small screw cap vials of ~1.9-ml. capacity. The vials are sealed, free of air, with a disk of polyethylene which is held in place by the cap. Titration of an aliquot of each sample under the nitrogen barrier at a suitable interval of time yields the standard curve which is given in Figure 4.

These procedures have been used for the assay of ribonuclease activity in samples from the column fractionation method of Hirs, Moore, and Stein (*1*) in various cellular fractions from pancreas, and in certain studies of inactivation of ribonuclease.

LITERATURE CITED

(1) Hirs, C. H. W., Moore, S., and Stein, W. H., *J. Biol. Chem.* 200, 493 (1953).

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Determination of Sulfur by Wet Combustion with Perchloric Acid

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Difficulties in the determination of sulfur in wood and pulp samples by the conventional method of Grote and Krekeler have been overcome by applying a wet combustion technique, using perchloric and nitric acids. The sulfur is reduced to hydrogen sulfide, by boiling with a reduction mixture, and determined iodometrically. The method gives quantitative yields for pure organic compounds and is suitable for a great variety of samples with sulfur contents from 0.01 to 100%. Sample weights can be varied from 9 mg. to 5 grams.

IN THIS institute the sulfur content of wood and pulp samples has hitherto been determined by the method of Grote and Krekeler (*4*). The sulfur content of these samples is sometimes fairly low, of the order of 1% or less, so that 1 gram or more is required for each analysis. This leads to lengthy combustions. In some samples the considerable ash content made the combustion tubes become opaque so that they had to be frequently replaced, leading to increased costs.

Because of these disadvantages it was decided to try wet combustion techniques for wood and pulp samples. First a method described by Klingstedt (*10*) was applied to pure organic substances, such as sulfosalicylic acid and thiourea; the results were often low and the length of time required for digestion, 2 to 4 hours, was considered disadvantageous. When the resulting solution was evaporated to dryness, a more or less black residue, obviously containing charred decomposition products, was sometimes obtained, an indication that the oxidation was not complete.

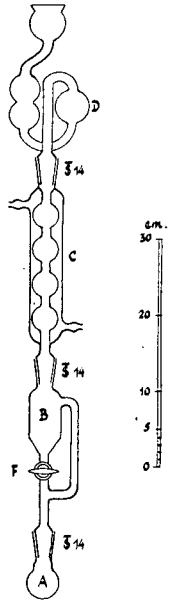

Figure 1. Wet decomposition apparatus

With this experience in mind, attention was turned to methods using perchloric acid for the wet combustion. Such methods for the determination of sulfur have been described by Kahane and coworkers, first in a method applicable to vulcanized rubber (*7*) and later for samples of biological origin (*11*). These methods were developed further by Wolesensky (*19*) and Smith (*17*) for the determination of sulfur in coal and in 1934 Kahane and Kahane (*9*) described a general method for determining sulfur in organic substances.

Preliminary experiments in this institute confirmed the observation of Kahane that sulfur-containing gases escape if the wet decomposition with perchloric acid is done in open vessels. The escaping gas is presumably sulfur dioxide, as no hydrogen sulfide could be detected.

According to Staudinger and Niessen (*18*), carbonyl sulfide may be formed in some cases. This substance is a relatively stable gas and it is possible that it escapes when the oxidation is carried out in an open vessel.

The experiments were continued with the apparatus previously described (*3*) (Figure 1). When this apparatus was used recoveries of sulfur were quantitative (see Tables III and IV), even when iodic acid was omitted, so it was considered superior to the one described by Kahane.

After the wet combustion the reaction flask contains a *mixture* of perchloric acid and the sulfuric acid to be determined, together with a small amount of nitric acid and the decomposition products of perchloric acid. After dilution with water it is possible to determine the sulfate content directly by precipitation with barium chloride solution. This procedure is, however, time-consuming and laborious and it would be more desirable to transform the sulfur into hydrogen sulfide. This may be accurately determined by volumetric or colorimetric methods which are much more sensitive than gravimetric methods for the determination of sulfate.

Boiling with a reducing mixture containing hydriodic acid as a method of reducing sulfates to sulfides was proposed by Lorant in 1929 (*12*), and this technique has been further developed for analytical purpose by Luke (*13*), Roth (*15*), and Johnson and Nishita (*5*). The method of Luke (*13*) for the determination of sulfur in rubber was of special interest because here reduction takes place in the presence of perchloric acid. This would appear to involve great risk of explosion. Experiments showed, however, that when the reducing mixture of Luke, which contains hydriodic, hypophosphorous, and hydrochloric acids, was boiled with perchloric acid in the proportion 3 to 1 there was no tendency to violent reaction; in fact, no reaction at all took place. When the experiment was repeated with mixtures containing more perchloric acid, a slow formation of iodine was obtained. It is obvious that if the reducing mixture is boiled with perchloric acid in the proportion 3 to 1, the temperature, about 120° C., is so low that the oxidizing properties of perchloric acid do not appear. In wet decomposition experiments the oxidizing properties of perchloric acid begin to be noticeable at about 150° to 180° C. Having carried out thousands of sulfur determinations, the author is convinced that there is no risk of violent reaction in boiling perchloric acid with the reducing mixture in the stated proportions.

When the reducing mixture was added directly to the mixture in the reaction flask after the wet decomposition, in order to boil off the hydrogen sulfide, some difficulties arose. Residual nitric acid and other oxidizing substances such as chlorine caused a formation of free iodine which reacted with the hydrogen sulfide already formed. Hydrobromic acid, which reduces nitric acid and other oxidizing substances formed without affecting the sulfuric or the perchloric acid, was found to be a most suitable reagent for destroying these substances. After this treatment the hydrogen sulfide could be boiled off without any formation of iodine.

The quantitative experiments on the reduction of sulfuric acid to hydrogen sulfide were carried out in the apparatus shown in Figure 2. In order to avoid any oxidation of the hydrogen sulfide formed, nitrogen, previously freed from oxygen by the method of Meyer and Ronge (*14*), was used as carrier gas. Although no abnormal results were obtained when nitrogen was used without prior purification, it seemed wise to standardize the purification procedure, since the quality of the nitrogen may vary in different gas cylinders. In order to reduce the amount of hydriodic and hydrochloric acids carried over into the receiver, water cooling was introduced, but it proved unnecessary to scrub the gas with water or dilute hydrochloric acid to remove acid fumes. The amount of these carried over during a 15-minute distillation is more than neutralized if the receiver contains 20 ml. of 3*N* sodium hydroxide solution. As the gases escaping from the apparatus are harmless, it can be operated on an open laboratory bench

Table I. Reduction of Potassium Sulfate without Additions

(Theoretical, 18.38% S)

Amount Taken, Mg.	S Found, %	Yield, %
9.96	18.37	99.95
10.10	18.39	100.05
11.19	18.41	100.16
11.96	18.39	100.05
11.71	18.39	100.05
11.40	18.43	100.27

Table II. Reduction of Potassium Sulfate

(Hydrobromic acid and perchloric acid added. Theoretical, 18.38% S)

Amount Taken, Mg.	Found, %	Yield, %
12.33	18.29	99.51
9.26	18.41	100.16
11.71	18.44	100.33
10.91	18.20	99.02
11.01	18.41	100.16
9.78	18.38	100.00

The design of the receiver and the titration of the hydrogen sulfide have been described (*2*).

To ensure quantitative recovery of sulfur with the reduction mixture of Luke, experiments were carried out in which dried potassium sulfate was boiled with 7 ml. of the reduction mixture in the apparatus mentioned. The results are given in Table I.

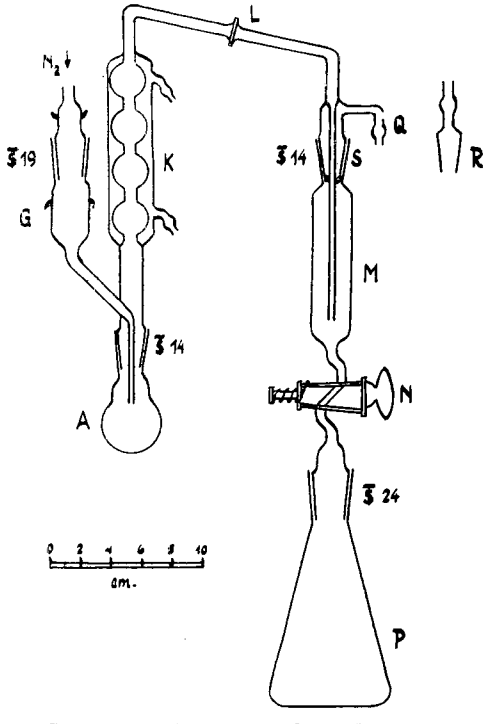

Figure 2. Apparatus for reduction

As these results were encouraging, similar experiments involving the addition of 2 ml. of perchloric acid and pretreatment with hydrobromic acid (see below) were carried out. In view of the success of these experiments (Table II), determinations of sulfur in organic substances were attempted. The final procedure for semimicro samples is given below. With this procedure the results became quantitative, the recoveries being between 98 and 101% (see Table IV).

Table III. Influence of Some Additions before Wet Decomposition

Variations. 1. According to procedure given for semimicro samples
2. Preoxidation with NaOH + H_2O_2
3. Preoxidation with Br_2 + H_2O_2
4. 500 mg. of glucose added
5. Ammonium chromate added

Variation	Substance	Amount Taken, Mg.	% S, Theoret.	% S, Found
1	Taurine	10.31	25.63	7.48
1		9.22		11.44
2		9.50		7.73
2		9.08		21.50
3		9.38		25.77
3		10.32		25.75
4		9.73		25.67
4		9.55		25.56
5		10.02		25.77
5		9.68		25.76
1	Sulfonal	11.98	28.09	26.23
3		10.82		25.29
3		9.33		26.83
4		9.32		26.13
5		10.04		28.20
5		13.20		28.12
5		13.59		28.25
1	Ba salt of sulfacetic acid	15.23	10.92	4.36
3		16.84		6.65
4		15.55		10.76
4		16.97		11.04
4		14.12		10.89
5		14.46		10.98
4 + 5		13.43		10.90

APPARATUS

Wet decomposition apparatus (Figure 1). For details, see (*2*).

Apparatus for determination of hydrogen sulfide (Figure 2). The apparatus is made of borosilicate glass with standard-taper joints; the ball and socket joint, *L*, has been introduced to reduce strains when handling the apparatus. The absorption vessel, *M*, and the titration flask, *P*, have been described (*2*). It is essential that the titration flask be made of extra-thick glass, to avoid implosions. The size of reaction flask *A* is not critical and for semimicro samples 50-ml. flasks can be used. When flask *P* is to be evacuated, mouthpiece *R* is connected to a water suction pump and inserted into joint *S*. The apparatus is easily cleaned by pouring acetone into funnel *G* and connecting *Q* to the suction pump.

Both sets of apparatus are obtainable from Firma Werner-Glas, Slöjdgatan 1, Stockholm C, Sweden.

REAGENTS

Acid mixture for wet decomposition, 40 ml. of perchloric acid, d = 1.69, and 60 ml. of nitric acid, d = 1.40.

Hydrobromic acid, d = 1.49.

Reduction mixture. Hydriodic acid (160 ml.), d = 1.70, 45 ml. of hypophosphorous acid (H_3PO_2) (50%), and 160 ml. of hydrochloric acid, d = 1.19, are refluxed for 1 hour. Nitrogen or carbon dioxide should be passed through the mixture during this operation.

Sodium hydroxide solution, 3*N*, 120 grams of sodium hydroxide per liter.

Phosphoric acid, 5*M*, phosphoric acid (342 ml.), d = 1.74, is diluted to 1 liter.

Iodine solution, about 0.01*N*.

Sodium thiosulfate solution, standardized 0.01*N*.

PROCEDURE FOR SEMIMICRO SAMPLES

The sample is weighed into a 50-ml. flask, *A*, provided with a No. 14 tapered joint. Five milliliters of the acid mixture for wet decomposition are introduced into the reflux receiver, *B* (Figure 1), with stopcock *F* closed. The condenser, *C*, the flask, *A*, and the scrubber, *D*, are mounted according to the figure. The scrubber is filled with a few milliliters of water or 3% hydrogen peroxide solution. Stopcock *F* is opened and, if necessary, flask *A* is heated with a microburner until nitrous fumes begin to form. After heating for 5 minutes, stopcock *F* is closed and heating is then continued until all the nitric acid has been collected in *B* and dense fumes of perchloric acid are formed in the reaction flask. The heating is moderated so that no fumes are allowed to pass through condenser *C*.

When the sample is completely oxidized, the apparatus is left for a few minutes to cool slightly. Stopcock *F* is opened and the

liquid in B is allowed to flow into A. The solution is boiled for a minute to rinse the apparatus, F is then closed, and most of the nitric acid is distilled into B until the first fumes of perchloric acid appear in A. The apparatus is then allowed to cool.

Reaction flask A is removed and 8 ml. of hydrobromic acid are added. The flask is heated gently while bromine gas is leaving the flask and until the contents have a light brown color. If a stream of inert gas is passed through the flask, this procedure is accelerated.

The apparatus for the determination of hydrogen sulfide (Figure 2) is now made ready. Fifteen milliliters of $5M$ phosphoric acid are introduced into titration flask P, and absorption vessel M with stopcock N open is inserted into the neck of the titration flask which is then evacuated through mouthpiece R. Stopcock N is closed, R is removed, and the titration flask with the absorption vessel, now filled with 20 ml. of $3N$ sodium hydroxide solution, is connected to the apparatus at S. Seven milliliters of the reduction mixture are added to the contents of flask A, which is immediately connected to the apparatus. A moderate stream of oxygen-free nitrogen is passed through the apparatus and the mixture in A is boiled for 15 minutes. All the hydrogen sulfide is now driven over and stopcock N is opened slowly, so that the adsorption solution flows down into the titration flask. Joint S is disconnected and M is rinsed with a little water which is also sucked down into P. Fifteen milliliters of iodine solution are pipetted into M and sucked down. After being rinsed, assembly MNP is shaken vigorously for 1 minute. If all the iodine is consumed, more is added and the shaking is repeated. When there is an excess of iodine in the titration vessel, the vacuum is released and absorption vessel M is removed. The excess iodine is back-titrated with standardized sodium thiosulfate solution, using starch solution as indicator.

CALCULATION

a = milliliters of thiosulfate solution consumed
b = milliliters of thiosulfate solution consumed by an amount of iodine solution equal to that added to the titration flask
n = normality of the thiosulfate solution
w = milligrams of sample taken
Percentage of sulfur. $\%\ S = 1603(b - a)n/w$

DISCUSSION

The main difference between wet combustion by this method and that of Kahane is the use of an apparatus which makes the addition of iodic acid unnecessary. In this apparatus the contact between the gases evolved during the combustion is better, so that the nitrogen dioxide initially formed fills the reflux receiver and remains there during the whole operation. All the sulfur dioxide, even that which may form during the later stages, coming into contact with the nitrogen dioxide forms sulfuric acid, which condenses on the inside walls of the apparatus and is brought down into the reaction flask during the second refluxing operation.

The time necessary for the complete oxidation of the sample depends on its nature but, in general, 10 minutes of boiling of the perchloric acid is sufficient. The reaction time can be determined if potassium chromate is added as an indicator, as proposed by Smith (*16*).

Some organic substances, usually of low molecular weight, have been reported as being resistant to oxidation by perchloric acid. Among sulfur-containing substances of this type Balks and Wehrmann (*1*) have found that taurine and thioglycollic acid are not completely oxidized by hot perchloric acid. They recommend that the substance be preoxidized by treatment with alkaline bromine solution or that a more easily oxidizable substance, such as starch, be added to the sample. Jones (*6*) has found that sulfonal is of the same type and that this substance is not oxidized even after pretreatment with aqua regia. The author has found that the barium salt of sulfoacetic acid is also of this type.

When the semimicromethod described was applied to these substances, in agreement with the previous authors it was found that the observed values for sulfur were low and irregular. Different methods of obtaining quantitative recoveries of sulfur were investigated and the results of these experiments are listed in Table III. Four variations (methods 2 to 5) of the basic procedure (method 1) were tried. Of special interest is the fact that the use of catalysts, as recommended by Smith (*16*), gives good results in all cases and the catalyst does not interfere with the conversion of sulfate into sulfide.

Theoretical values were obtained when trimethylenethiourea, which gives carbonyl sulfide on oxidation with nitric acid (*18*), was determined according to this method: (theory, 27.59; found, 27.39 and 27.66%). This shows that this type of substance can be analyzed without special precautions.

When the sulfur content of inorganic substances is to be determined it is sometimes unnecessary to carry out the wet combustion. Sulfates, sulfites, and sulfides give up all their sulfur as hydrogen sulfide on boiling with the reduction mixture, but elementary sulfur does not. In the case of sulfites and thiosulfates an alkaline preoxidation is recommended to avoid losses due to formation in the cold of large quantities of sulfur dioxide.

The presence of barium in the sample does not involve any difficulty, although it may sometimes be wise to increase reduction time to 30 minutes. However, in the case of inorganic samples it is better to treat the sample with 2 ml. of hot perchloric acid before reduction in order to dissolve the barium sulfate. When the reduction mixture is added the barium sulfate is reprecipitated in a finely divided form, which reacts easily with the reduction mixture. After reduction is complete there should be no precipitate left in the reaction flask.

Some typical determinations on diverse pure substances, organic and inorganic, are given in Table IV.

Table IV. Typical Examples, Analysis of Pure Samples

Substance	Amount Taken, Mg.	% S, Theoret.	% S, Found	Yield, %
Sulfosalicylic acid	21.89	12.62	12.66	100.3
	22.21		12.66	100.3
Thiourea	19.92	42.13	42.13	100.0
	22.69		41.60	98.7
l-Cystine	20.43	26.70	26.71	100.0
	20.59		26.56	99.5
Mercaptobenzthiazole	18.09	38.34	38.92	101.5
	19.48		37.99	99.1
p-Toluenesulfonylchloride	22.46	16.82	16.69	99.2
p-Bromosulfonylanisidine	28.57	9.37	9.43	100.6
	22.62		9.42	100.5
1(4-Methoxyphenyl)-propane-2-sulfonic acid, Ba salt	19.12	10.76	10.82	100.6
1-Phenyl-3-hydroxypropane-2-sulfonic acid, Ba salt	14.82	11.29	11.32	100.3
Sulfur	9.03	100.0	101.4	101.4
Barium sulfate	20.15	13.72	13.62	99.2
Potassium sulfate, see Tables I and II				
Benzyl disulfide	60.00	26.03	25.69	98.7
	11.84		25.89	99.5
s-Benzylthiouronium chloride	12.50	15.82	15.87	100.3
	10.69		15.91	99.9
Phenylthiourea	11.38	21.07	21.10	100.1
	11.69		21.02	99.8

BIOLOGICAL AND TECHNICAL SAMPLES

The main purpose of this investigation was not to find a new semimicrotechnique for the determination of sulfur in pure, organic substances, since many such methods exist. However, the fact that the recoveries of sulfur are quantitative for a great number of pure compounds shows that the method can be safely applied to substances of biological and technical origin.

When technical or biological materials are to be analyzed, the sample weight often has to be increased for two reasons: The sulfur content may be rather low, of the order of 1% or less, or the material may not be homogeneous, so that a sample of 0.5 gram or more must be taken in order to get reproducible results.

It is on products of this kind that the wet combustion technique would be expected to have many advantages compared with dry combustion methods. However, the procedure cannot be made as general as the one for semimicro samples and it must be varied to suit the individual case. An injudicious use of perchloric acid may lead to violent reaction but if some simple rules are followed, this risk can be eliminated.

The reaction of perchloric acid with organic matter has been

Table V. Some Examples, Analysis of Technical Products

Substance	Amount Taken, Mg.	% S, Found	% S, Expected
Rubber D-0	60.07	0.831	0.79
Rubber D-1	66.89	0.785	0.79
Rubber D-2	90.40	0.782	0.79
Poly(methyl methacrylate)	1081	0.064	0.06
	945.5	0.063	0.06
Leather 031154	194.77	1.61	1.50
	136.90	1.61	1.50
Calves hair	263.0	3.77	3.66
Light concrete	69.28	2.12	2.4
	69.40	2.10	2.4
Mortar, weathered	98.54	1.25	..

studied by Smith (*16*), and in a recent paper by Kahane (*8*) a review is given of its applications in analytical chemistry. In both these papers the risks attending the use of perchloric acid are discussed. In the special case of sulfur determination it seems safe to decompose the sample with a mixture of nitric and perchloric acid, as the nitric acid destroys all the easily oxidizable matter, leaving only a residue of material more resistant to the perchloric acid. When nitric acid is used, the temperature of the acid mixture rises slowly and smoothly, so that the oxidizing power of the perchloric acid is gradually increased, thereby effecting a progressive breakdown of the sample. If a new substance or material is to be investigated a small sample, 50 mg. or so, should be decomposed by the semimicromethod and if the decomposition proceeds without charring or excessive foaming the sample weight can be increased. If charring occurs, the amount of nitric acid can be increased or the time for reflux boiling prolonged. The use of catalysts according to Smith (*16*) may speed up the procedure appreciably.

For example, when samples of wood are to be decomposed, 5 ml. of the acid mixture and an additional 5 ml. of nitric acid are used for decomposition. The reaction flask is heated a little until a reaction starts, giving rise to large amounts of nitrous gases. When this reaction has subsided, the sample is dissolved and the procedure given for semimicro samples is followed. When the nitric acid is almost driven over into the reflux collector, the flame is moderated so that the perchloric acid does not react too rapidly, thereby driving fumes of perchloric acid out of the apparatus.

These directions for wood samples can be followed with only minor modifications for most types of plant material. The amounts of hydrobromic acid and reduction mixture should be chosen so that the proportions remain the same as those in the *semimicroprocedure. However, if extra nitric acid is added,* there is no need to increase these quantities, as the amount of perchloric acid left in the reaction flask is unchanged.

The method has also been applied to a few samples of technical products of other kinds. Samples of leather, coal, rubber, plastics, hair, light concrete, and mortar were analyzed. Some of the results are listed in Table V. These samples have been analyzed mainly as described for wood samples; in the case of concrete and mortar, the same results were obtained if the wet decomposition was omitted. The method should be suitable for all materials that can be decomposed by wet decomposition. In the case of aqueous solutions it is wise to add some hydrogen peroxide to the sample, so that neither hydrogen sulfide nor sulfur dioxide may escape before the concentration of nitric acid has reached an oxidizing level.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Balks, R., and Wehrmann, O., *Bodenkunde u. Pflanzenernähr.* **5**, 48 (1937).
(2) Bethge, P. O., *Anal. Chim. Acta* **9**, 129 (1953).
(3) *Ibid.*, **10**, 317 (1954).
(4) Grote, W., and Krekeler, H., *Angew. Chem.* **46**, 106 (1933).
(5) Johnson, C. M., and Nishita, H., ANAL. CHEM. **24**, 736 (1952).
(6) Jones, J. H., *J. Assoc. Offic. Agr. Chemists* **26**, 182 (1943).
(7) Kahane, E., *Caoutchouc gutta percha* **24**, 13549 (1927).
(8) Kahane, E., *Österr. Chem.-Ztg.* 55, 209 (1954).
(9) Kahane, E., and Kahane, M., *Bull. soc. chim.* **1**, 280 (1934).
(10) Klingstedt, F. W., *Z. anal. Chem.* **112**, 101 (1938).
(11) Lematte, L., Boinot, G., and Kahane, E., *Compt. rend. soc. biol.* **96**, 1211 (1927).
(12) Lorant, I. S., *Z. physiol. Chem.* **185**, 245 (1929).
(13) Luke, C. L., IND. ENG. CHEM., ANAL. ED. **15**, 602 (1943).
(14) Meyer, F. R., and Ronge, G., *Angew. Chem.* **52**, 637 (1939).
(15) Roth, H., *Mikrochemie ver. Mikrochim. Acta* **36–37**, 379 (1951).
(16) Smith, G. F., *Anal. Chim. Acta* **8**, 397 (1953).
(17) Smith, G. F., and Deem, A. G., IND. ENG. CHEM., ANAL. ED. **4**, 227 (1932).
(18) Staudinger, H., and Niessen, G., *Chem. Ber.* **9**, 1223 (1953).
(19) Wolesensky, E., *Ind. Eng. Chem.* **20**, 1234 (1928).

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Titration of Gallium with Ferrocyanide

Application of Dead-Stop End Point

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Dilute solutions of gallium(III) salts as low as 0.001*M* may be titrated with potassium ferrocyanide using the dead-stop end point. The optimum conditions for the titration were determined to be 230 mv., pH = 2.0, and 50° C. The presence of ammonium ion, various acids, and pH were studied. The stoichiometry of the reaction corresponds to the formation of gallium ferrocyanide, $Ga_4[Fe(CN)_6]_3$.

A METHOD for determination of gallium in dilute aqueous solution employs the dead-stop end point apparatus of Foulk and Bawden (*1*). It consists of titrating gallium(III) solutions with potassium ferrocyanide to form gallium ferrocyanide, $Ga_4[Fe(CN)_6]_3$, which is a white precipitate. As long as any gallium(III) is present in solution, the galvanometer remains near zero, but as soon as any excess ferrocyanide ion appears in solution, a large deflection occurs. A potentiometric method has been described by Kirschman and Ramsey (*2*).

EXPERIMENTAL

Standard solutions of gallium nitrate and potassium ferrocyanide were prepared from reagent grade materials. The potassium ferrocyanide was recrystallized as the trihydrate and weighed directly to make standard solutions. The gallium was weighed as the metal, dissolved in nitric acid, and diluted to volume. All other chemicals employed were reagent grade.

Dilute solutions of gallium nitrate were titrated at about 50° C. and with a potential difference between the platinum

wire electrodes of 230 mv. Above 300 mv. the end points became erratic and with the potential difference much below 200 mv. the galvanometer deflections became too small to yield clearly defined end points. The optimum temperature was chosen to be 50° C. because the reaction was too sluggish at room temperature (26° C.) and, at 75° C., the end points became erratic. However, even at 50° C. the reaction was not instantaneous, as immediately after each addition of ferrocyanide there was a large initial deflection of the galvanometer which decreased to a small steady value after about a minute. The galvanometer employed had a sensitivity of 1.7×10^{-8} ampere per millimeter.

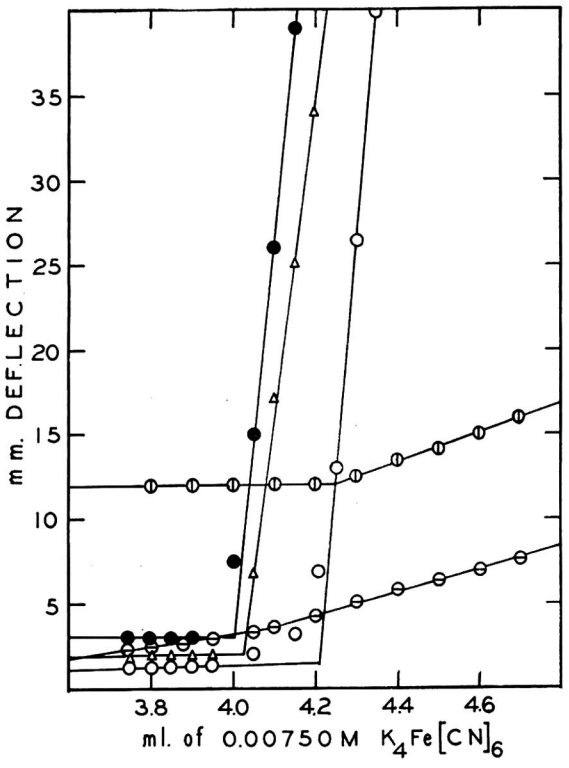

Figure 1. Titration curves of 0.0100*M* $Ga(NO_3)_3$ with 0.00750*M* $K_4Fe(CN)_6$

● NH_4^+ absent, pH 2.0 with H_2SO_4
○ NH_4^+ present, pH 2.0 with H_2SO_4
⊖ NH_4^+ absent, 0.75*M* with H_2SO_4
⊕ NH_4^+ absent, 1.2*M* with $HClO_4$
Δ NH_4^+ absent, pH 2.0 with $HClO_4$
In all cases 4.00 ml. of 0.0100*M* $Ga(NO_3)_3$ were titrated with $K_4Fe(CN)_6$

Enough acid was added to the gallium solutions to maintain a pH of about 2.0 during the titrations. The addition of a little acid seemed to make the galvanometer deflections at the end point larger and also eliminated the danger of precipitating gallium hydroxide. Acids, with different anions, were employed in some titrations, and, in a few cases, higher concentrations were used.

Because of the similarity of this titration and that of zinc(II) with ferrocyanide, experiments were conducted in the presence of ammonium ion to see whether it affected the titration curves markedly as in the case of zinc (*3*).

RESULTS

In Table I are typical results of the titration of 0.0100*M* gallium(III) nitrate with 0.00750*M* potassium ferrocyanide in the presence and absence of ammonium ion. Also shown is the stoichiometric ratio, *R*, which is the ratio of the volume of ferrocyanide to that of the gallium solution at the end point. In all cases the potential difference was 230 mv., the temperature approximately 50° C., and the pH about 2.0.

The average value of *R* for 31 titrations with no ammonium ion was 1.005 and the average deviation was 6 parts per thousand. For 39 titrations with about 0.02*M* ammonium sulfate present in the gallium solutions, the average value of *R* was 1.057 and the average deviation was 6 parts per thousand.

In the case of zinc, the presence of ammonium ion is necessary in order to obtain sharp end points (*3*). In the present case, end points are obtained in either the presence or absence of ammonium ion, but about 5% more potassium ferrocyanide solution is required to reach the end point when the gallium solution contains about 0.02*M* ammonium ion than when none is present. Since the theoretical stoichiometric ratio should be unity on the basis of the concentrations of gallium nitrate and potassium ferrocyanide, it appears desirable to titrate in the absence of ammonium ion if possible.

The effect of acidity on the titration curve was studied by titrating potassium ferrocyanide into a gallium solution which was 0.75*M* with sulfuric acid and into another which was 1.2*M* with perchloric acid. In both cases the sharpness of the end point was considerably reduced (as indicated in Figure 1). However, the end points were sharp when hydrochloric, sulfuric, or perchloric acid was employed to adjust the pH of the gallium solution to about 2.0, and the stoichiometry was unchanged.

Two experiments giving identical results indicated that it is possible to titrate 0.00750*M* potassium ferrocyanide with 0.0100*M* gallium nitrate, if the time between the addition of the potassium ferrocyanide solution to the acid solution at 50° C. and the commencement of titration with gallium is not more than a few minutes. Upon waiting for longer times, apparently the ferrocyanide ion decomposes in warm acid, because the solution and the gallium precipitate become discolored and the end points are erratic.

When the gallium nitrate concentration was reduced to 0.001*M* and titrated with 0.00075*M* potassium ferrocyanide, the average deviation for three titrations was 6% and the average deviation for five titrations of 0.0001*M* gallium(III) nitrate with 0.00011*M* potassium ferrocyanide was 10%. In both cases the gallium solutions were 0.02*M* in ammonium ion.

Table I. Titration of 0.0100*M* Gallium Nitrate with 0.00750*M* Potassium Ferrocyanide

$Ga(NO_3)_3$, Ml.	$K_4Fe(CN)_6$, Ml.		*R*[a]	
	NH_4^+ present	NH_4^+ absent	NH_4^+ present	NH_4^+ absent
5.00	5.28	5.00	1.056	1.000
5.00	5.25	5.04	1.055	1.008
10.00	10.50	10.02	1.050	1.002
10.00	10.51	10.00	1.051	1.000
15.00	15.70	15.00	1.047	1.000
15.00	15.72	15.03	1.048	1.002
17.00	17.87	17.00	1.051	1.000
17.00	17.90	17.01	1.053	1.001
		Av.	1.051	1.002
		Av. deviations	0.002	0.002

[a] *R* is ratio of volumes of ferrocyanide to gallium at each end point.

The interference of other cations was not studied, but presumably the presence of any other cations which form insoluble salts with ferrocyanide ion would cause difficulty.

In summary, it is possible to titrate gallium(III) with ferrocyanide in very dilute acid solutions using the dead-stop end point, but to obtain reproducibility better than 1%, the conditions must be maintained constant.

LITERATURE CITED

(1) Foulk, C. W., and Bawden, A. T., *J. Am. Chem. Soc.* **48**, 2045 (1926).
(2) Kirschman, H. D., and Ramsey, J. B., *Ibid.*, **50**, 1632 (1928).
(3) Swinehart, D. F., ANAL. CHEM. **23**, 380 (1951).

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Electronic Conversion for Graphic Recording with the Chevenard Photographically Recording Thermobalance

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The postwar commercial availability of the Chevenard automatic photographically recording thermobalance has given a great impetus to the heretofore inadequately exploited thermoanalytical technique of thermogravimetry. Because of the inconveniences of photographic recording techniques, a method has been developed for obtaining a pen-and-ink recording of the thermogravimetric curves with a potentiometric-type recorder. This is accomplished by means of a linear variable differential transformer used as the transducer, and does not require modification of the balance.

SINCE the introduction of thermoanalytical techniques utilizing a thermobalance, reported by Honda (*7*) in 1915, several manually operated instruments have been used in studies of materials such as clays, minerals, metallurgical specimens, and many inorganic and organic compounds. In 1935, Dubois (*3*) reported the first automatic photographically recording thermobalance; but it was not until the post-World War II years that Chevenard (*2*) developed the first commercially available recording thermobalance. With this apparatus Duval and others critically evaluated a multitude of precipitates which have been proposed for, and applied to, gravimetric analysis. These investigations (*4*) have demonstrated the value of this formerly neglected technique as a research and analytical tool for the study of high temperature reactions and their kinetics.

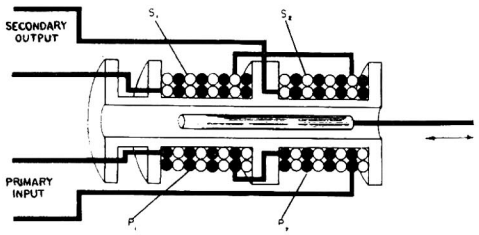

Figure 1. Linear variable differential transformer

The Chevenard thermobalance (*5*) makes use of an automatic photographic technique for continuously recording the weight of the system under investigation. (A mechanical recording system has recently been made available as optional equipment for this apparatus.) However, photographic recording methods are inherently limited and involve inconveniences such as the need for a dark room in which the camera can be loaded and the photographic paper processed; the working space required for the photographic housing and camera; the inability to observe the curves during the course of the reaction under study; unavailability of the record until the photographic processing and drying are completed; a lack of reference grid lines on the record; the lengthy setup time required for each determination; the limitation of camera drum speeds; and the time-consuming procedure involved in the occasional realignment of the light source, balance-beam mirror, and camera drum. Therefore, an electronic conversion of the balance was considered in order to provide direct pen-and-ink recording on potentiometric recorders. A suitable transducer to convert balance-beam displacements into electrical signals has to be accurately linear, lightweight, frictionless in operation, sensitive to very small deflections, and relatively inexpensive, and involve only a minimum modification of the balance. The device finally selected was a linear variable differential transformer, used in conjunction with a demodulator, which satisfactorily met all these requirements. Peterson (*8*) has independently reported the application of this transducer to the conversion of any analytical balance to an automatic recording microbalance.

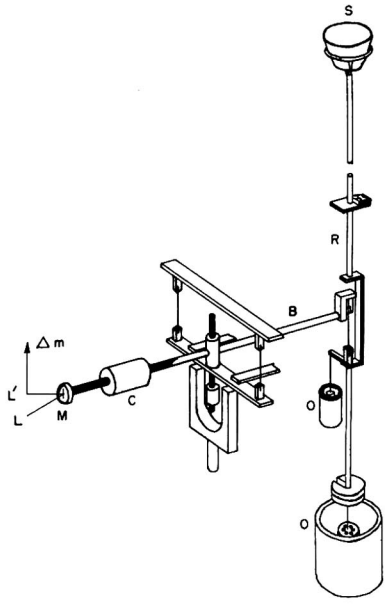

Figure 2. Diagram of Chevenard thermobalance

B. Balance beam
C. Counterpoise
M. Mirror
O. Oil pots
R. Support rod
S. Sample crucible

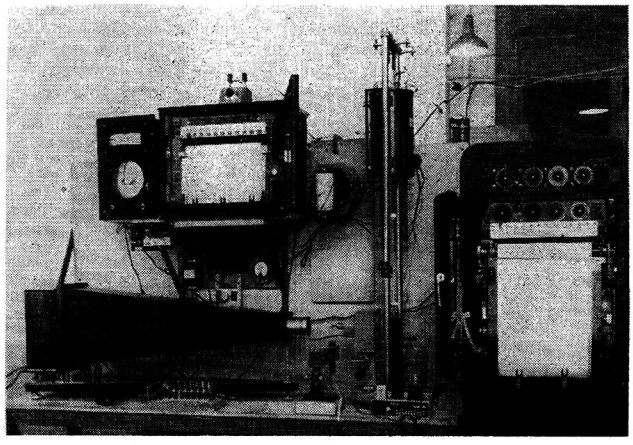

Figure 3. Chevenard thermobalance, temperature controlling and recording equipment, photographic and electronic recording apparatus

Table I. Thermogravimetric Analysis of Calcium Nitrate Tetrahydrate

Assumed Product	% Loss		
	Theoretical	Observed	Difference
$Ca(NO_3)_2.2H_2O$	15.2	14.4	−0.8
$Ca(NO_3)_2$	30.5	30.3	−0.2
CaO	76.2	76.3	+0.1
CaO (dry basis)	65.9	66.0	+0.1

DESCRIPTION OF APPARATUS

Transducer. A linear variable differential transformer is a transducer that generates an alternating current signal which is directly proportional to the linear displacement of the armature from the electrical center (null point) of the transformer windings (coil). The device (*1*), shown in Figure 1, consists of a primary coil, two secondary coils, and an armature of magnetic material. The primary coil, P_1, P_2, is energized from a suitable sinusoidal source; the two secondary coils, S_1, S_2, are connected so that their output voltages are 180° out of phase; and the armature is located so that it can alter the relative flux distribution which exists between the primary coil and the two secondary coils. Motion of the armature toward secondary coil, S_1, results in an increased output of one phase, and motion towards S_2 produces an increased output 180° out of phase. If S_1 and S_2 are identical and the armature is centrally located so that each receives an equal amount of flux, the voltages induced in these secondary coils will be equal and out of phase and a theoretical output of zero will result. This condition represents the null, or balance point, of the differential transformer. The transformer with armature shown in Figure 1 is the Class 6206-A Atcotran differential transformer with a linear range of ±0.5 inch and an accuracy within at least ±0.5%. The armature used on the thermobalance was purchased without a shaft and then mounted on a silica tube slightly longer than the armature. The demodulator used to rectify the alternating current signals generated by the transformer is a vibrator-type phase-discriminating converter, Atcotran Type 6101-C.

Balance. The balance (Figure 2) consists of a wire-supported beam, B, to one end of which is attached a vertical rod, R, supporting the sample, S, and to the other end a mirror, M. The mirror is used to reflect a light beam, LML^1, onto a photographic paper wrapped around a synchronous motor-driven drum. The light beam deflections are linearly proportional to the change in weight of the sample, ΔM, and thus a curve is obtained of the changes in weight as a function of time. Oil pots, O, are used to minimize oscillations of the balance beam.

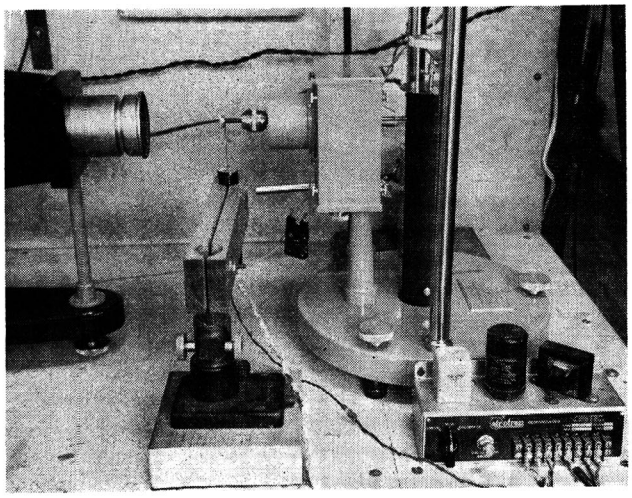

Figure 4. Close-up of thermobalance showing electronic conversion

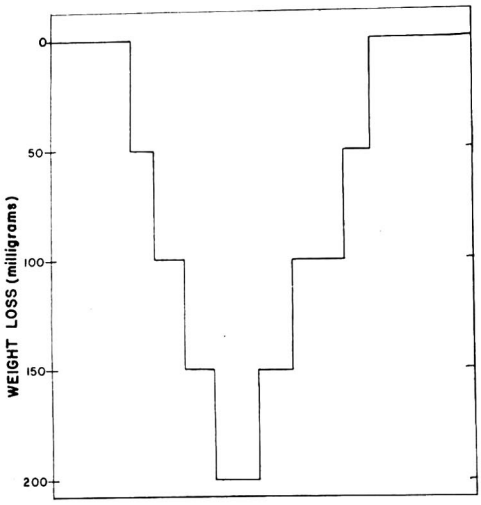

Figure 5. Incremental weight changes as recorded with electronically converted thermobalance

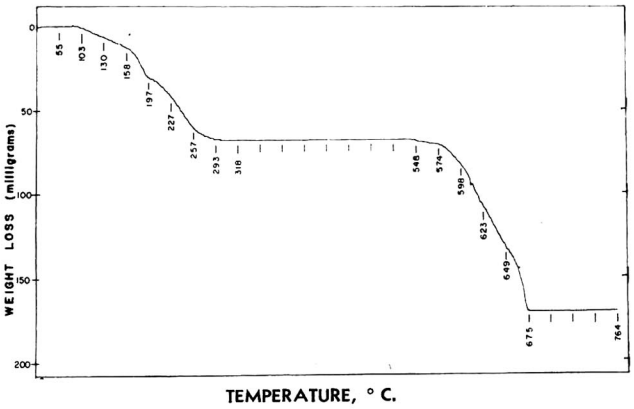

Figure 6. Thermogravimetric analysis of calcium nitrate tetrahydrate (0.2228 gram), 15°/min.

Figure 3 is a picture of the Chevenard thermobalance with both the photographic and electronic recording units, and equipment for programming and recording the temperature of the furnace. Figure 4 is a close-up view of the balance as modified for electronic recording, wherein the armature of the differential transformer is suspended from the balance beam by a thread so that it hangs freely within the transformer coil.

The silica tube, instead of the conventional steel shaft, was used for mounting the armature to reduce the weight which must be placed on the balance beam. It was found that the critical design of the balance causes nonlinearities in the beam displacements when the armature is added. This was compensated for by turning down the counterpoise on a lathe and bringing it up to the desired weight by wrapping it with nickel wire. The transformer coil is supported and vertically positioned by a wooden clamp mounted on a rack-and-pinion mechanism from a micrometer slide comparator. The nonmetallic clamp is used to ensure linear operation of the transformer. The demodulator, which is operated from a 110-volt, constant voltage regulator, supplies the 6-volt, 60-cycle signal required for the primary coil and feeds the rectified direct current secondary voltage into the potentiometric strip chart recorder. The recorder used is a Leeds & Northrup Speedomax with adjustable zero and adjustable range.

OPERATION OF BALANCE

With the sample in its container positioned on the support rod, and a fractional weight equal to one half the desired weight range

placed on the calibration platform, the beam is balanced in a horizontal position by means of the counterpoise. This corresponds to the mid-point of the deflection for the full scale change in weight over this range. The coil is positioned with the rack and pinion, so that the armature is at the null point and a zero voltage is obtained. The recorder is then adjusted so that the voltage indicated is equivalent to the mid-point of the full scale change in weight to be measured. In this way the most linear portions of the balance beam and the differential transformer are used for measuring and recording the weight changes. The calibration consists of placing another equal fractional weight on the platform to obtain the voltage output equivalent to zero weight loss, and then removing both weights to obtain the recorder point corresponding to a full scale loss in weight. Replacing the fractional weights restores the balance and the recorder pen to the position of zero loss in weight, and the balance is now ready for the determination which is conducted in the normal manner. For reactions involving a gain in weight, the same calibration procedure is followed using a reversed polarity of the transducer output.

If recorders with adjustable zero and adjustable range are not available, conventional electronic potentiometric instruments with a range of about 10 mv. can be used (*6*) with a battery and variable limiting resistor in series with the differential transformer to provide a bucking voltage for obtaining adjustable zero positioning of the pen. A precision potentiometer may be used as an external voltage divider for variable range adjustment.

The range of weight changes that can be linearly recorded is essentially the 400 mg. that can be obtained photographically, and the accuracy over a range of 200 mg. appears to be that involved in reading the record—i.e., approximately 0.25%. The stability, using a source of constant line voltage, is 0.25% over a period of 8 hours; and the reproducibility over a range of 200 mg. is 0.5 mg. or 0.25% of the scale, whichever is greater, as indicated by the stepwise addition and removal of fractional weights shown in Figure 5. The response time is that of the balance—namely, 2 seconds for a 200-mg. change in weight.

A typical thermogravimetric curve obtained with the electronically converted Chevenard thermobalance, at a heating rate of 15° C. per minute, is illustrated by calcium nitrate tetrahydrate shown in Figure 6. The calculated and observed changes in weight are summarized in Table I.

LITERATURE CITED

(1) Automatic Temperature Control Co., Inc., Philadelphia, Pa., Bull. **R-31**, Eng. Data Sheet No. **73**.
(2) Chevenard, P., Waché, X., De La Tullaye, R., *Bull. soc. chim.*, (5) **10**, 41 (1944).
(3) Dubois, P., thesis, University of Paris, No. 2428, June 26, 1935.
(4) Duval, C., "Inorganic Thermogravimetric Analysis," Elsevier Publishing Co., Amsterdam, 1953.
(5) Ferner Co., Inc., R. Y. (American Agents for Chevenard thermobalance), Malden 48, Mass., Brochure.
(6) Gordon, S., and Campbell, C., *Bull. Am. Ceram. Soc.*, in press.
(7) Honda, K., *Science Repts. Tôhoku Imp. Univ.* **4**, 97 (1915).
(8) Peterson, A., *Instruments and Automation* **28**, 1104–6 (1955).

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Unsaturation Determination by Acid-Catalyzed Bromination

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In an attempt to develop a bromination procedure which would be more widely applicable to the determination of unsaturation in organic compounds, the original method of Kaufman has been investigated and modified. The sample is reacted with a solution of bromine and excess sodium bromide in methanol-water medium containing a small amount of hydrochloric acid catalyst. The excess bromine is determined by conversion to iodine and titration with sodium thiosulfate. The reagent reacts quantitatively and rapidly with a variety of unsaturated compounds. Errors due to substitution are minimized and in most cases results are unaffected by extended reaction time. Correlation between reactivity of the unsaturated compounds and molecular structure is discussed.

HALOGENATION procedures used in these laboratories for the determination of unsaturation include the Wijs (*8*) method using iodine chloride, the Hanus (*2*) method employing iodine bromide, bromine in carbon tetrachloride with a mixed catalyst as described by Braae (*1*), and a modified Francis (*4*) method using an acidified solution of potassium bromate-bromide. These reagents are limited in their usefulness because of inaccuracies and unpredictable side reactions. Their use in most cases requires standardized procedures for specific concentrations of the unsaturated compound.

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Kaufman (*3*, *6*) reported satisfactory results using a solution of bromine in methanol saturated with sodium bromide. It has been stated that bromine added to a solution of sodium bromide forms the probable complex

$$Br_2 + NaBr \rightleftharpoons NaBr.Br_2$$

which is a mild brominating reagent exhibiting little tendency towards substitution. It reacts with unsaturates according to the equation:

$$R{-}CH{=}CH{-}R + NaBr.Br_2 \rightarrow R{-}CHBrCHBr{-}R + NaBr$$

Investigation in this laboratory (*5*) has shown that the solubility of sodium bromide in methanol is insufficient to provide the excess bromide ion necessary to complex the bromine completely. As pointed out by Uhrig and Levin (*7*), without excess sodium bromide, use of the reagent is subject to serious error because of substitution. Addition of saturated aqueous sodium bromide to the reaction mixture prior to the introduction of the sample was tried and this modification has been used for some time. Substitution error was greatly reduced in many instances. However, in those cases where scrupulous care was not observed in the addition of the aqueous sodium bromide solution, reproducibility was noted to be adversely affected. Examination has shown that this lack of reproducibility may be caused by small variations in sodium bromide concentration or the quantity of aqueous sodium bromide solution used. A more uniform reaction mixture less subject to poor reproducibility was obtained by

Table I. Titer of Sodium Thiosulfate Required by 50 Ml. of Bromine-Bromide Reagent

(Time in hours *vs.* Ml. 0.1*N* reagent)

Hours	Ml.	Hours	Ml.
1	49.97	24	49.75
2	50.00	47	49.44
3	50.00	72	49.24
4	49.97	96	49.32
5	49.96	192	49.08
6	49.96	384	47.74
7	49.96		

incorporation of water, additional sodium bromide, and a reduction in bromine concentration in the reagent. Values of the titer of standard sodium thiosulfate required for blank determinations over a period of 384 hours are shown in Table I.

The use of catalysts for bromine addition has been reported by Braae (*1*) and others. In general, halogen acids, mercury, silver, nickel, and antimony salts, as well as mixtures of these and other components, have been successful in accelerating addition. Hydrochloric acid was chosen as the catalyst in this investigation both because of its ready accessibility and the greatly increased reaction velocities exhibited with its use. It has been incorporated in the bromine-bromide reagent without loss of stability.

REAGENTS

Bromine-Bromide Reagent, approximately 0.1*N* in bromine. Transfer 2.5 ml. of reagent grade bromine to a 1-liter flask containing 300 ml. of distilled water, 300 ml. of methanol, 100 grams of sodium bromide, and 10 ml. of concentrated hydrochloric acid. Dilute to 1 liter with additional methanol and mix thoroughly. The reagent may be used immediately after preparation. The use of pressure is recommended in pipetting bromine-bromide reagent, which may be accomplished by fitting a flask with a two-hole rubber stopper and inserting a 50-ml. pipet through one hole so that the tip extends below the surface of the liquid. Through the other hole fit a piece of glass tubing to which is attached a rubber atomizer bulb. Pressure on the atomizer bulb then causes the pipet to fill with the reagent.

Sodium Bromide, reagent grade crystals

Potassium Iodide, 15% aqueous solution

Sodium Thiosulfate, 0.1*N* aqueous standardized against acidified potassium iodate

PROCEDURE

To each of a sufficient number of 250-ml. glass-stoppered Erlenmeyer flasks to make all sample and blank determinations in duplicate, add 5 to 10 grams of sodium bromide crystals. Carefully pipet 50 ml. of approximately 0.1*N* bromine-bromide reagent into each of the flasks, filling the pipet by pressure. Reserve at least two flasks for blank determinations. Into each of the other flasks introduce the sample which should contain not more than 3.5 meq. of unsaturated compound. Weigh the sample to the nearest 0.1 mg., using a suitable weighing pipet or hypo-

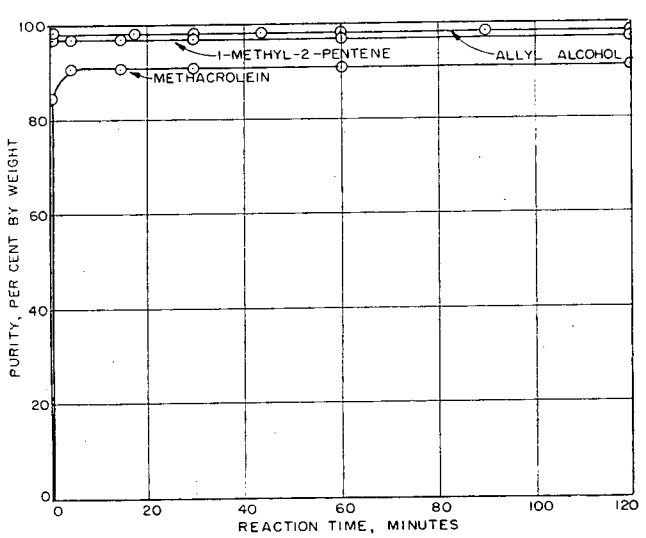


Figure 1. Typical reaction rate curves with acid-catalyzed bromination reagent

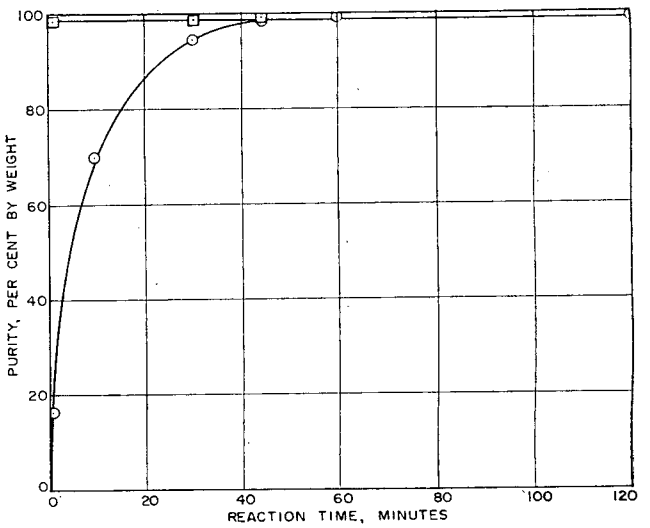


Figure 2. Styrene reaction rate curves

Comparison of acid-catalyzed and uncatalyzed reagent

⊡ Acid-catalyzed
⊙ Uncatalyzed

Table II. Analysis of Organic Compounds by Bromination with Acid-Catalyzed Reagent

Compound	Weight Per Cent Theoretical	Weight Per Cent Observed[a]	Reaction Time, Min.
Acrolein (acrylaldehyde)	99.8[b]	99.7 ± 0.1[c] (11)	30–120
	99.4[b]	99.2 ± 0.1 (6)	
	99.5[b]	99.5 ± 0.2 (7)	
Allyl alcohol	98–100[d]	98.1 ± 0.1 (3)	10–120
	95–100[d]	96.9 ± 0.1 (3)	
Allyl chloride (3-chloropropene)	98.5[e]	98.0 ± 0.0 (4)	15–60
Allylidene diacetate	99.1[f]	98.6 ± 0.3 (6)	180
Cinnamic alcohol (cinnamyl alcohol)	97.4[g]	97.4 ± 0.3 (4)	5–120
Crotonaldehyde	98.7[h]	96.6 ± 0.1 (4)	5–120
	75–100[d]	60.3 ± 0.1 (3)	
1-Decene	95.2[g]	95.2 ± 0.2 (4)	30–60
Diallyl maleate	94.0[f]	92.7 ± 0.2 (4)	15–120
2,5-Dimethyl-1,5-hexadiene	103.4[g]	103.4 ± 0.2 (5)	30–120
3,5-Dimethyl-3-hexen-5-olide	99.0[f]	99.6 ± 0.3 (5)	15–120
2-Ethylcrotonaldehyde	99.4[h]	99.2 ± 0.3 (5)	20–30
4-Ethyl-2-octenal	99.0[g]	99.0 ± 0.2 (4)	120
2-Ethyl-3-propylacrolein	98.7[h]	98.3 ± 0.1 (4)	15–90
3-Ethyl-3-propylacrylic acid	95.2[i]	95.0 ± 0.1 (3)	45–60
2-Formyl-3,4-dihydro-2*H*-pyran	98.6[h]	99.5 ± 0.1 (6)	5–120
2-Heptene	96.7[j]	97.1 ± 0.1 (3)	1–120
2-Hexenal	98.0[h]	98.1 ± 0.1 (3)	15–30
	90.2[g]	90.2 ± 0.1 (6)	
Mesityl oxide (4-methyl-3-penten-2-one)	99.6[g]	99.6 ± 0.1 (4)	15–30
Methacrolein (methacrylaldehyde)	91.0[h]	90.9 ± 0.1 (4)	15–120
4-Methyl-1-pentene	95–100[d]	92.6 ± 0.2 (3)	10–60
4-Methyl-2-pentene	95.1[j]	96.1 ± 0.8 (3)	15–60
2-Octene	95.1[j]	95.1 ± 0.3 (3)	1–120
1-Propene-1,3-diacetate	98.1[g]	98.1 ± 0.0 (5)	15–120
	97.8[f]	97.0 ± 0.1 (4)	
Styrene	99.0[k]	99.1 ± 0.1 (4)	1–120
Tetrahydrobenzaldehyde	100.0[h]	100.4 ± 0.2 (4)	1–60
Vinyl acetate	98.5[f]	98.5 ± 0.1 (4)	1–120
Vinyl cyclohexene	89.9[g]	89.7 ± 0.2 (8)	1–120
Vinyl propionate	96.6[j]	96.5 ± 0.1 (7)	1–120

[a] Numbers in parentheses indicate number of determinations.
[b] Mass spectrometer.
[c] Average deviation.
[d] Estimated.
[e] Chlorine analysis.
[f] Saponification.
[g] Estimated from reaction rate curves with acid-catalyzed bromine-bromide reagent.
[h] Reaction with hydroxylamine hydrochloride.
[i] Acidimetric titration.
[j] Reaction with mercuric acetate reagent.
[k] Estimated from physical properties.

dermic syringe. If a dilution of the sample is to be used, add to the blanks an amount of solvent equal to the volume of the aliquot taken. Dilutions in methanol were used in this investigation for those sample sizes below 0.1 gram by taking 5- or 10-ml. aliquots. Precautions must be taken to prevent the loss of volatile samples during weighing operations. Solvents suitable for use as diluents are methanol, ethyl alcohol, acetonitrile, diisopropyl ether, acetic acid, and water. When water is used as a diluent or if the sample contains appreciable water, add the sample to the reaction vessel and saturate with sodium bromide before adding the bromine-bromide reagent.

Allow the samples and blanks to stand together at room temperature for the minimum time specified in Table II. (Essentially the same result is obtained by using the maximum time.) To each flask in turn, add 50 ml. of methanol and 10 ml. of 15% potassium iodide solution. Titrate immediately with standard 0.1*N* sodium thiosulfate just to the disappearance of the yellow iodine color. The difference in titration between a blank and a sample is a measure of the unsaturated compound.

Table III. Effect of Varying Water Concentration in Brominating Reagent on Apparent Purities of Unsaturated Compounds

Compound	Purity, Wt. %	Water Concentration, %					
		0	10	20	30	40	50
		Purity obtained, wt. %					
3,5-Dimethyl-3-hexen-5-olide	99.6	44.7	72.2	94.7	99.7	99.8	99.6
Vinyl propionate	96.5	96.5	96.4	96.3	96.4	...	...
Tetrahydrobenzaldehyde	100.0	100.1	100.0	99.6	100.0	...	...
Allyl chloride	98.5	61.4	90.0	97.9	98.8	98.6	...

DISCUSSION

A bromine-bromide reagent prepared as described is stable, and the use of special bromination vessels to prevent the loss of bromine is unnecessary (see Table I). The optimum water concentration in the reagent is approximately 30%. Water concentrations below 30% produced erratic results with some compounds while concentrations up to 50% had little effect except to reduce the solubilities of some substances in the reagent. The change in the apparent purity of several compounds with change in water concentration is shown in Table III. Erratic results were obtained in every case when sodium bromide concentration was less than 10%. Increases in the concentration up to 50% had no effect. The reagent contains 10% sodium bromide, which is sufficient to maintain excess bromide ion during reaction using 10-ml. or smaller aliquots. Hydrochloric acid was varied from 0.4 to 2% with no apparent effect.

Interference was encountered when isopropyl alcohol, isobutyl alcohol, hexyl alcohol, acetone, tetrahydropyran, dioxane, and dimethyl Cellosolve were used as solvents. In general, their presence in relatively low concentrations in the sample, however, caused no serious error. The extent of interference should be determined for each case. Any easily oxidizable compound, such as acetaldehyde, may interfere when present in sufficiently high concentration.

In the investigation of compounds listed in Table II, determinations were made over a range of reaction times. In most cases 2 hours was arbitrarily selected as the maximum practical reaction time. The time interval during which quantitative results have been obtained is also shown. Reaction rate curves, apparent purity plotted against reaction time in minutes, were plotted. Wherever practical, purities were obtained by independent methods. Figure 1 illustrates curves obtained for three of the compounds investigated. From the essentially horizontal upper portion of the curves, it is evident that there were virtually no secondary substitution or oxidation reactions.

Increased reaction rate exhibited by catalysis with hydrochloric acid is shown in Figure 2, comparing reaction rate curves of styrene using both a catalyzed and an uncatalyzed reagent. The same concentration of reagents and the same sample dilution were used. This contrast in reaction rates is not so marked in every case, but some increase was noted for each compound investigated.

Variation in sample size has no appreciable effect upon the determination of unsaturation. In most cases 1 meq. of excess bromine was provided; however, good results were obtained when less than this amount was present in excess.

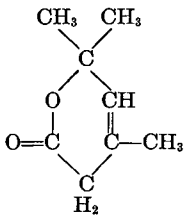
The several types of compounds investigated indicate some correlation between reactivity and molecular structure. Those classes of compounds which do not brominate satisfactorily are listed in Table IV. Straight-chain olefins below the nonenes in molecular weight brominate easily. Chain branching appears to inhibit bromination, although some branched-chain olefins below the heptenes have been determined. Isolated multiple bond systems in hydrocarbons have been determined as in the case of 2,5-dimethyl-1,5-hexadiene, whereas the conjugated system in 2,5-dimethyl-2,4-hexadiene could not be determined.

The addition of bromine to carbon-carbon unsaturation adjacent to a carboxyl group is slow, and the inhibition becomes more pronounced with the introduction of more electronegative alkyl groups replacing acidic hydrogen. The effect is greater with esters of such dibasic acids as maleic and fumaric where less than 10% bromination occurred after 120 minutes. On the other hand, bromine addition is enhanced by halogen or hydroxyl groups in a position beta to the double bond as in allyl chloride and allyl alcohol.

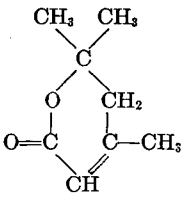
Conjugation with the ketonic carbonyl group inhibits bromination with somewhat anomalous results. Reaction with 2,8-dimethyl-3,6-nonadien-5-one, $CH_3CH(CH_3)CH{=}CHC{=}OCH{=}CHCH(CH_3)CH_3$, is 85% complete in 5 hours. From the reaction rate curve it appears that one double bond reacts completely in several minutes, whereas the second resists addition because of the electron attracting carbonyl group. Bromination of butylideneacetone, $CH_3CH_2CH_2CH{=}CHC{=}OCH_3$, is accompanied by some apparent oxidation. 5-Ethyl-3-nonen-2-one, $CH_3CH_2CH_2CH_2CH(C_2H_5)CH{=}CHC{=}O{-}CH_3$, gives erratic results which approach quantitative addition. These interferences are apparently caused by the acetyl group which may react with the reagent. Conjugation with the ketonic carbonyl group within nonaromatic ring structures also inhibits bromination. This is illustrated by the bromination of 3,5-dimethyl-3-hexene-5-olide (I) which is quantitative in 15 minutes (see structural formulas I and II);

Table IV. Classes of Compounds Which Cannot Be Determined Satisfactorily with Acid-Catalyzed Bromination Reagent

Olefins	Carbonyls
2-Butyl-1-octene	Allylacetone
2,5-Dimethyl-2,4-hexadiene	Butylideneacetone
Diisobutylene	2,8-Dimethyl-3,6-nonadien-5-one
Dodecene	5-Ethyl-3-nonen-2-one
Tetradecene	Furfural
Triisobutylene	2,4-Hexadienal
Conjugated Unsaturated Acids and Esters	Double Bonds Adjacent to Ether Linkages
Crotonic acid	Cyclohexenyl acetate
Crotonic anhydride	Divinyl Carbitol
Dibutyl maleate	Monovinyl Carbitol
Diethyl fumarate	Vinyl 2-chloroethyl ether
Diethyl maleate	Vinyl 2-ethylhexyl ether
3,5-Dimethyl-2-hexen-5-olide	Vinyl tetradecyl ether
Endomethylenetetrahydrophthalic anhydride	Vinyl undecyl ether
Ethyl acrylate	
2-Ethylhexyl crotonate	
Methyl acrylate	



I



II

whereas 3,5-dimethyl-2-hexene-5-olide (II) shows no tendency to brominate after 2 hours. Aliphatic aldehydic carbonyl groups have little effect when conjugated with the carbon-carbon double bond except, perhaps, to speed addition of bromine.

Double bonds adjacent to ether linkages are not consistent in their behavior toward bromine-bromide reagent. Vinyl alkyl ethers cannot be determined because of erratic results, probably because of hydrolysis, whereas 2-formyl-3,4-dihydro-2*H*-pyran reacts quantitatively.

ACKNOWLEDGMENT

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LITERATURE CITED

(1) Braae, B., ANAL. CHEM. **21**, 1461 (1949).
(2) Hanus, J., *Chem. Zentr.* **2**, 1217 (1901).
(3) Kaufman, H. P., "Studien auf dem Fettgebiet," Verlag Chemie, Berlin, 1935.
(4) Mulliken, S., and Wakeman, R., IND. ENG. CHEM., ANAL. ED. **7**, 59 (1935).
(5) Sutherland, W. W., and Quinn, J. H., Carbide and Carbon Chem. Co., South Charleston, W. Va., unpublished data, July 12, 1942.
(6) Trappe, W., *Biochem. Z.* **296**, 174 (1938).
(7) Uhrig, K., and Levin, H., IND. ENG. CHEM., ANAL. ED. **13**, 90 (1941).
(8) Wijs, J. J. A., *Ber. deut. chem. Ges.* **31**, 750 (1898).

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Volumetric Determination of Selenium

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The volumetric permanganate procedure of Schrenk and Browning has been extended to the determination of selenium in refined selenium, sodium selenite, sodium selenate, and iron selenide.

THE conventional gravimetric procedure of determining selenium by precipitation of elemental selenium with sulfur dioxide is subject to errors in washing and ignition which render the procedure unsuitable for the accurate estimation of selenium in refined selenium and selenium compounds. Of the various volumetric procedures for the determination of selenium, the iodometric methods of Evans (*2*), Deshmukh and Sant (*1*), and Schulek and Koros (*7*) all require carefully controlled conditions, some of which are difficult to maintain. The reproducibility of these procedures, as well as Stamm and Goehring's permanganate method (*8*), left much to be desired.

A commendable permanganate method which has received scant attention is that of Gooch and Clemons (*3*) as extended by Schrenk and Browning (*6*). In this method selenium and tellurium are oxidized in sulfuric acid medium from the quadrivalent to the sexivalent state by an excess of permanganate, the excess being determined by back-titration with ferrous ammonium sulfate. Disodium phosphate is added to prevent the precipitation of manganese dioxide. This paper extends Schrenk and Browning's procedure to the estimation of selenium in refined selenium, sodium selenite, sodium selenate, and iron selenide.

PROCEDURE

Selenium, Sodium Selenite, and Sodium Selenate. Weigh accurately a 1-gram sample into a 300-ml. Berzelius beaker. Dissolve sodium selenite in 50 ml. of warm distilled water, and dilute to the mark in a 500-ml. volumetric flask. Treat refined selenium with 20 ml. of mixed acid water, nitric acid, sulfuric acid (1:1:1), and allow to simmer under the boiling point. When all the nitrogen oxides have been driven off and the solution has turned colorless, cool the solution and dilute to the mark in a 500-ml. volumetric flask.

Run from a buret a 25-ml. aliquot of the sample solution (50 ml. for sodium selenite) into a 250-ml. Erlenmeyer flask. Acidify with 20 ml. of 18*N* sulfuric acid, dilute with 100 ml. of distilled water, and add 12 grams of disodium phosphate ($Na_2HPO_4 \cdot 7H_2O$). Stir the solution to dissolve the phosphate and add from a buret 20 ml. of 0.1*N* potassium permanganate solution. Let the solution stand for 30 minutes to complete the oxidation. Back-titrate the excess of permanganate with 0.1*N* ferrous ammonium sulfate. Add one drop of *o*-phenanthroline ferrous sulfate indicator near the end point, which is detected by the color change from pale blue to bright red.

Dissolve sodium selenate in 15 ml. of 18*N* sulfuric acid at moderate heat. Cool the solution and make up to the mark in a 250-ml. volumetric flask. Take a 25-ml. aliquot in a 250-ml. Erlenmeyer flask and add 1 gram of hydroxylamine hydrochloride. Boil gently for 10 minutes until all the selenium has precipitated. Cool and dissolve the selenium in 20 ml. of the mixed acid added cautiously to avoid violent reaction. Heat just under the boiling point to expel nitrogen oxide fumes. Now proceed with the cool solution in the same manner as for aliquots for selenium and sodium selenite samples.

Iron Selenide. Dissolve a 1-gram sample by adding gradually 30 ml. of hot mixed acid. Boil gently to dissolve the last particles of iron selenide and keep boiling until the first crystals of ferric sulfate have precipitated. Dilute the cooled solution with 50 ml. of distilled water, mix to dissolve the salts, transfer to a 250-ml. volumetric flask, and make up to the mark. Titrate a 25-ml. aliquot in the manner previously described.

It is recommended that the titer of the permanganate and ferrous ammonium sulfate solutions be checked carefully with each set of determinations by duplicate analyses of high-purity selenium.

DISCUSSION

Refined Selenium. Commercial selenium powder generally analyzes around 99.5% selenium, with tellurium as the principal impurity. A correction for the tellurium content of the sample may be realized by Schrenk and Browning's dichromate method (*5*) or by direct permanganate titration following volatilization of the selenium by sulfuric acid fuming at elevated temperature (*4*). Following the procedure detailed, the selenium values reported in Table I were obtained. The impurity analyses are given to indicate clearly the composition of the samples. The superiority of the volumetric procedure is realized when it is noted that a careful gravimetric determination of samples 1, 2, 3,

Table I. Analysis of Refined Selenium Powder

Sample	Se, %	Te, %	SiO_2, %	Cu, %	Pb, %	Fe, %	Hg, %	Sb, %	S, %
1	99.65	0.30	0.077	0.0014	0.012	0.021	0.010	0.0046	Nil
2	99.64	0.26	0.029	0.0010	0.017	0.017	0.0009	0.0033	Nil
3	99.65	0.23	0.014	0.0017	0.013	0.024	0.007	0.0033	Nil
4	99.49	0.23	0.024	0.0039	0.017	0.018	0.0005	0.0039	Nil

and 4 gave the values 98.73, 99.15, 98.81, and 98.73% selenium, respectively.

Sodium Selenite. The permanganate procedure applied to sodium selenite was found to give selenium values in good agreement with the theoretical value, 45.65%. Typical samples analyzed 45.45 and 45.67% selenium.

Sodium Selenate. As no convenient means is available for reducing sexivalent selenium to the quadrivalent state, the most satisfactory procedure of analyzing sodium selenate by the permanganate method was found to be reduction of the selenate to elemental selenium by hydroxylamine hydrochloride, followed by oxidation to the quadrivalent state by nitric acid. Typical analyses gave 41.84, 41.77, and 41.84%, values agreeing well with the theoretical value, 41.79%.

Iron Selenide. The selenium in iron selenide may be determined readily by the permanganate method following dissolution of the sample in hot mixed nitric-sulfuric acid. In order to check the selenium value indirectly, the iron content of a number of samples was determined by hydrolytic precipitation from a separate aliquot of the sample solution following the removal of selenium and tellurium. A typical analysis gave selenium 58.49%, iron 40.49%, and tellurium 0.47%. The theoretical selenium figure is 58.57%.

ACKNOWLEDGMENT

The authors are indebted to Herbert Marshall for the determination of the impurities in the refined selenium samples.

LITERATURE CITED

(1) Deshmukh, G. S., and Sant, B. R., *Analyst* **77**, 272 (1952).
(2) Evans, B. S., *Ibid.*, **67**, 346 (1942).
(3) Gooch, F. A., and Clemons, C. F., *Am. J. Sci.* **50**, 51 (1895).
(4) Marshall, H., Canadian Copper Refiners, Ltd., unpublished work.
(5) Schrenk, W. T., and Browning, B. L., *J. Am. Chem. Soc.* **48**, 139 (1926).
(6) *Ibid.*, p. 2550.
(7) Schulek, E., and Koros, E., *Z. anal. Chem.* **139**, 20 (1953).
(8) Stamm, H., and Goehring, M., *Ibid.*, **120**, 230 (1940).

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Coulometric Determination of Organic Bases in Acetonitrile

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The coulometric method of analysis is extended to the determination of amines in essentially nonaqueous solution. Nonaqueous solvents have been shown to be most useful in the determination of these weaker bases. The solvent employed is acetonitrile containing 0.05*N* lithium perchlorate trihydrate. The water content of this solution is approximately 0.3%. Hydrogen ion is produced by anodic oxidation of the water and the ion detected by the conventional glass-calomel electrode combination. Pyridine, diphenylguanidine, triethylamine, and benzylamine have been titrated in milligram quantities; the usual error is less than 2%. Aromatic amines tested by this method cannot be titrated. They apparently undergo partial or complete oxidation by the oxygen in solution which is produced concurrently with hydrogen ion at the anode.

THE value of coulometry as a method for the determination of small amounts of materials has been amply demonstrated in recent years (*2*). DeFord (*4*) has applied this method for the titration of acids and bases in aqueous systems. Carson and Ko (*1*) have titrated acids coulometrically in a 70/30 mixture of isopropyl alcohol and water.

Extensive work (*6, 7*) has shown, however, that weak acids and bases are most easily titrated in nonaqueous solutions. An extension of coulometry to essentially nonaqueous systems should then be useful in the determination of small amounts of these compounds. Coulometry has a further advantage in that it is readily adapted to automatic procedures. The present paper deals with a coulometric method for determining weak bases in acetonitrile.

The usual source of hydrogen ion in the aqueous coulometric titrations for base is the solvent itself. Acetonitrile, however, is not oxidized to yield hydrogen ion anodically—the reaction water so readily undergoes. The small amount of water required in the solvent as the source of hydrogen ion was introduced in the form of a hydrate of lithium perchlorate. A 0.05*N* solution of lithium perchlorate trihydrate in acetonitrile served as the solvent for the determinations. The water content of this solution is 0.3%.

A potentiometric titration curve for pyridine dissolved in ace-

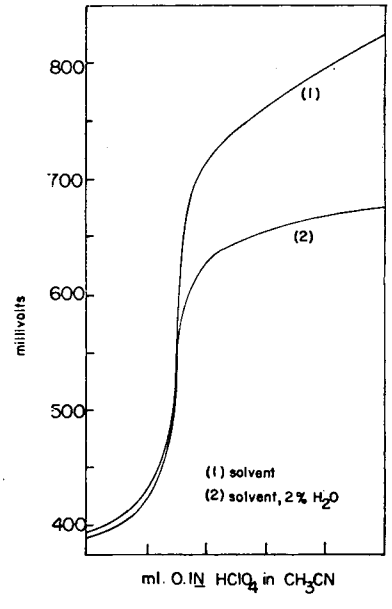

Figure 1. Titration of pyridine in acetonitrile solution

Table I. Results on Four Amines

Compound	Sample, Mg.	Current, Ma.	Time, Sec.	Result, Mg.	Error, Mg.	% Error
Pyridine	0.581	2.044	346.8	0.581	0.000	0.0
		2.041	353.4	0.591	0.010	1.7
		2.039	351.0	0.587	0.006	1.0
		2.039	349.8	0.585	0.004	0.7
		1.984	364.2	0.592	0.011	1.9
		1.986	361.8	0.589	0.008	1.4
		1.992	350.4	0.572	−0.009	−1.5
		1.998	352.8	0.578	−0.003	−1.0
				Av. 0.584	±0.006	±1.0
1,3-Diphenyl-guanidine	1.400	2.018	316.8	1.400	0.000	0.0
		1.976	324.0	1.396	−0.004	−0.3
		1.998	319.2	1.401	0.001	0.1
		1.994	320.4	1.398	−0.002	−0.1
				Av. 1.399	±0.002	±0.1
Triethyl-amine	1.092	2.044	512.4	1.097	0.005	0.4
		2.056	509.4	1.097	0.005	0.4
		2.064	503.4	1.089	−0.003	−0.3
		2.070	501.0	1.087	−0.005	−0.4
				Av. 1.092	±0.005	±0.4
Benzylamine	0.638	1.897	304.2	0.640	0.002	0.3
		1.894	301.8	0.634	−0.004	−0.6
		1.894	289.8	0.609	−0.029	−4.5
		1.999	296.4	0.657	0.019	3.0
		2.005	286.2	0.637	−0.001	−0.2
		2.012	283.8	0.634	−0.004	−0.6
				Av. 0.635	±0.010	±1.5

tonitrile containing 2% of water is shown in Figure 1, together with the titration curve for pyridine in the pure solvent. Although the end-point potential is lowered for the solution containing water, a clearly defined titration break is apparent, and titration values are identical. Coulometric titrations in a solvent containing only 0.3% of water should not then offer end-point problems.

APPARATUS

The generation apparatus and end-point detection system are of conventional design, similar to that described by Cooke (*2*). Essentially constant current is obtained from two 45-volt dry cells connected in series through a 33,000-ohm resistor. Current is measured by determining the voltage drop through a precision resistor of 24.00 ohms with a standard potentiometer. Measurement of current during the course of a number of determinations indicated that the current variation was of the order of 3 or 4 parts per thousand.

A large platinum gauze electrode immersed in the body of the solution served as the generator anode. The cathode was a platinum wire spiral isolated in a separate compartment containing an aqueous 1% solution of the lithium salt. The two compartments were connected by a salt bridge of 1% lithium perchlorate trihydrate, 5% agar. The resistance of the bridge decreases the current passed through the solution, but this is necessary, as when the cathode was immersed in the acetonitrile solution constant generating currents could not be obtained. These bridges are slowly dehydrated by the acetonitrile solution and must be replaced from time to time.

The indicating system consisted of glass and calomel electrodes, a Leeds & Northrup line-operated pH meter, and a Speedomax recorder. The recorder's speed can be varied to 1 or 2 inches of strip chart per minute and gives a full scale deflection of 350 or 700 mv. The recorder was used in place of a clock, since its speed is precise. Time can be measured to within 0.3 second, which is 1 part per thousand on a 300-second run. Studies were also made using a Beckman Model G pH meter and a time clock reading to 0.1 second. Comparable results were obtained. All values reported, however, were obtained from the recorded data.

Titrations were carried out in a 150-ml. tall-form beaker. Solutions were stirred with a magnetic stirrer. All electrodes were contained in a large rubber stopper fitted to the beaker.

Recorder, pH meter, stirrer, and coulometer control box were all grounded to eliminate stray voltages, and the potentiometer was shielded with aluminum foil. Generator and potentiometer leads were of shielded wire. Shielding and grounding appear to be essential for these titrations, as the detection system is extremely sensitive to voltage transients.

REAGENTS

The acetonitrile used in this work is commercial grade obtained from the Carbide and Carbon Chemicals Co. The perchlorate salt is manufactured by the G. Frederick Smith Chemical Co. The amines were reagent grade chemicals and were tested for purity by conventional nonaqueous titration with perchloric acid.

Samples were prepared by dissolving weighed quantities of the amines in acetonitrile and serially diluting to the desired value. The supporting solution was made by dissolving 8 grams of lithium perchlorate trihydrate in 1 liter of acetonitrile and filtering.

PROCEDURE

The procedure is essentially the same as that described by Cooke, Reilley, and Furman (*3*). One hundred milliliters of 0.05*N* base solution is placed in the 150-ml. beaker, the solution is stirred, and the recording is begun. Current is allowed to flow until the indicator potential shows a value between 300 and 350 mv. (near the inflection point of the blank curve). Without stopping the generation current a 2.0-ml. sample of amine solution is pipetted into the beaker.

The indicator potential falls immediately to a lower value, and then rises again to the end-point potential as the generation proceeds. Generation current is measured during this interval. When the indicator potential has again risen to its previous value, another sample is added to the same solution and the procedure is continued. Five to six samples may be added to the original solution in this manner during one run.

The time elapsed to completion of the titration is determined by measuring the distance between points of equal potential on the upward sloping curve of the recording, and multiplying by the appropriate factor. Final results are obtained by multiplying current, time, and the appropriate factor in milligrams per microcoulomb for the amine under consideration.

As Cooke, Reilley, and Furman (*3*) have pointed out, measurements need not be made at the exact end-point value of a titration, as long as they are made at point of equal indicator potential for both blank and sample, if this value is reasonably near the true end point. Data collected during this investigation showed that time measurements made of the same sample 100 mv. apart were virtually identical.

RESULTS

A recording of the indicating electrode potential with time, obtained during hydrogen ion generation in the acetonitrile solution, is illustrated in Figure 2. Generation current was 2.073 ± 0.005 ma. The inflection point of the curve is between 300 and 350 mv.

Results obtained on the four amines determined are listed in Table I together with error in milligrams and per cent error.

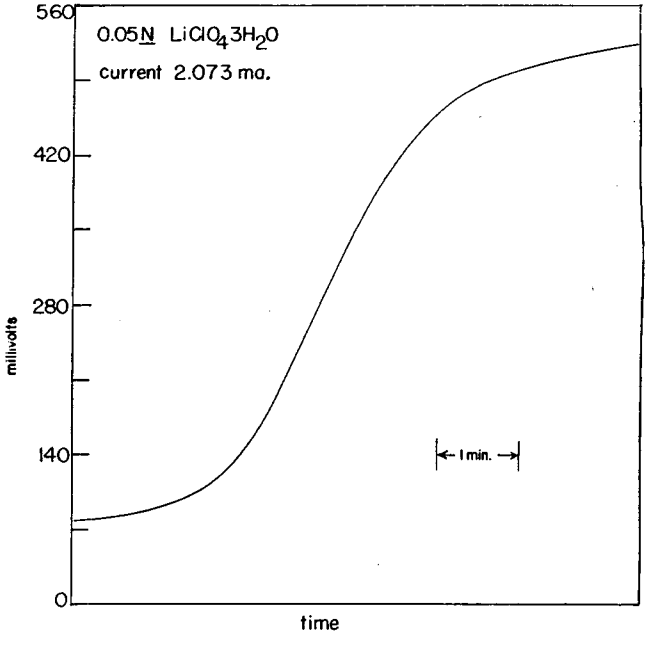

Figure 2. Coulometric generation of hydrogen ion in acetonitrile solution

The first value in any group of runs is not included, as this result was invariably lower than any of the following values. Average errors run less than 2% for any compound and individual errors are also less than this value, except for two of the values for benzylamine. In most cases an error of 1 second in determining the elapsed time of titration from reading the recordings would be equal to 2 or 3 γ of amine if the current were about 2 ma.

Attempts were also made to determine several aromatic amines by the same procedure. None of the compounds tested (diphenylamine, *p*-toluidine and phenylenediamine) gave quantitative results.

When samples of diphenylamine were added to the pretitrated solvent, the entire solution turned violet; this color eventually faded to yellow brown. No break in the indicator current was noted when the sample was added. This behavior is believed to be due to oxidation of the amine by oxygen generated in solution at the anode concurrently with hydrogen ion.

Fieser and Fieser (*5*) state that diphenylamine is readily oxidized by chemical means to tetraphenylhydrazine. This may be the reaction undergone in this solution.

If the amine and perchloric acid are mixed in acetonitrile, no color is exhibited. The color reaction occurs in pretitrated solution, however, even after the generating current has been turned off. The reaction cannot then be direct oxidation of the amine at the anode.

Phenylenediamine and *p*-toluidine both give breaks in the indicator current, but results are very low, indicating that partial oxidation has occurred. The phenylenediamine solution turns bright yellow during the course of the reaction.

Caution must be exerted in making sure that chlorides are not present in solution when hydrogen ion is generated. Chloride is evidently oxidized to chlorine in acetonitrile and high results will be obtained. The solution also turns yellow with prolonged generation if chlorides are present.

ACKNOWLEDGMENT

The author gratefully acknowledges the assistance of Everett W. Hobart in constructing the generation system.

LITERATURE CITED

(1) Carson, W. N., and Ko, R., ANAL. CHEM. 23, 1019 (1951).
(2) Cooke, W. D., "Coulometric Methods, Organic Analysis," vol. II, pp. 169–93, New York, Interscience, 1954.
(3) Cooke, W. D., Reilley, C. N., and Furman, N. H., ANAL. CHEM. 23, 1662 (1952).
(4) DeFord, D. D., Pitts, J. N., and Johns, C. J., *Ibid.*, 23, 938 (1951).
(5) Fieser, L. F., and Fieser, M., "Organic Chemistry," pp. 610–11, Heath, Boston, 1944.
(6) Fritz, J. S., "Acid-Base Titration in Nonaqueous Media," G. Frederick Smith Chemical Co., Columbus, Ohio, 1952.
(7) Pifer, C. W., Wollish, E. G., and Schmall, M., *J. Am. Pharm. Assoc.* 42, 509 (1953).

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Separation of 2,4-Dinitrophenylhydrazones of Aldehydes and Ketones by Paper Chromatography

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A convenient and highly sensitive paper chromatographic method for separating homologs of low molecular weight 2,4-dinitrophenylhydrazones is described. Using phenoxyethanol-impregnated paper as the stationary phase and heptane as the mobile phase it has been found possible to obtain good separation of as much as 250 γ of 2,4-dinitrophenylhydrazones in a single spot.

VARIOUS methods have been described for the separation of 2,4-dinitrophenylhydrazones of aldehydes and ketones by paper chromatography (*1, 2, 4*). However, many of these systems possess serious limitations in ability to separate homologs and have other inconvenient features. Some require especially impregnated paper which has to be prepared by difficultly reproducible techniques. In others, the quantity of 2,4-dinitrophenylhydrazone is limited to such small amounts that detection of the spots becomes uncertain. Attempts to increase the quantity of 2,4-dinitrophenylhydrazone in such systems leads to streaking and poor separation.

Neher and Wettstein (*3*) have described a two-phase paper chromatographic system for separating steroid compounds. This system, using phenoxyethanol-impregnated paper as the stationary phase and heptane as the mobile phase, has been found to be well adapted to the separation of homologs of low molecular weight 2,4-dinitrophenylhydrazones. As much as 250 γ of a 2,4-dinitrophenylhydrazone in a spot 1 cm. in diameter can be chromatographed with no appreciable streaking. The spots obtained with such amounts of material are readily visible without spraying or use of ultraviolet light. Also, as all solvents used are easily evaporated, the separated 2,4-dinitrophenylhydrazones can be readily eluted and recovered in pure state from the paper.

EXPERIMENTAL

Descending development of the paper chromatograms was used. Strips of Whatman No. 7 filter paper were dipped in a solution of 10% phenoxyethanol in acetone, blotted free of excess solution, and then dried in air for a few minutes. The 2,4-dinitrophenylhydrazones, dissolved in methanol, were applied to the paper to give spots 0.5 to 1 cm. in diameter. The paper strips

Table I. Mobilities of 2,4-Dinitrophenylhydrazones

2,4-Dinitrophenylhydrazone	Melting Point, ° C.	Mobility in 20 Hours, Cm. from Origin	Weight, γ
Formaldehyde	167	5.5	200
Acetaldehyde	169	9.0	200
Propionaldehyde	156	12.0	200
Butylaldehyde	122	15.5	200
Valeraldehyde	106	22.0	200
Heptaldehyde	108	29.0	200
Acetone	122	13.5	200
2-Butanone	117	20.5	200
2-Pentanone	144	26.5	200
3-Pentanone	156	28.5	200
4-Hydroxy-4-methyl-2-pentanone	202	21.5	200
Benzaldehyde	239	Streaked	50
Anisaldehyde	256	Streaked	50
Veratraldehyde	265	2.0	50

Solvents. Stationary phase, phenoxyethanol.
Moving phase, heptane saturated with phenoxyethanol.
Paper, Whatman No. 7
Development, descending.

were then suspended from a trough in a chromatographic jar, saturated with heptane. The moving phase, heptane saturated with phenoxyethanol, was added to the trough and the jar was sealed.

Twenty hours of development time were found to be adequate for most separations (see Table I). However, the time can be extended or shortened if necessary. The size of the separated spots was 2 to 3 cm. in diameter. Inasmuch as the solvent runs off the end of the paper in the time required for most separations, R_f values cannot be used for identification of the spots. It is necessary therefore to use standard substances for comparison. Where substances of widely differing mobilities are present in the same mixture, the eluate from the paper can be collected and rechromatographed.

LITERATURE CITED

(1) Kirchner, J. G., and Keller, G. J., *J. Am. Chem. Soc.* **72**, 1867 (1950).
(2) Kostir, J. V., and Slavik, K., *Collection Czechoslov. Chem. Communs.* **15**, 17 (1950).
(3) Neher, R., and Wettstein, A., *Helv. Chim. Acta* **35**, 276 (1952).
(4) Rice, R. G., Keller, G. J., and Kirchner, J. G., ANAL. CHEM. **23**, 194 (1951).

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Colorimetric Determination of Decaborane Using *N,N*-Diethylnicotinamide

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A method for the direct determination of decaborane is based upon the formation of an orange-red solution (maximum absorption 430 to 500 mμ) with decaborane and *N,N*-diethylnicotinamide. Such solutions follow Beer's law from 20 to 240 γ of decaborane per cc. Boric acid, boron salts, protein solutions, diborane, and pentaborane cause no interference with the procedure. The method is applicable to decaborane in aqueous systems as well as in cyclohexane.

IN CONNECTION with some biochemical studies with decaborane, a convenient method of determining this compound was desired. Because of the known affinity of the boranes for nitrogen (*3*, *4*), the possible use for this purpose of the reaction of decaborane with nitrogen heterocyclic compounds was investigated. Of a variety of such compounds, many gave evidence for reaction with decaborane and two, *N*-*N*-diethylnicotinamide and nicotinamide, formed stable orange-red solutions. The color developed with decaborane and *N,N*-diethylnicotinamide was found applicable to a quantitative estimation of decaborane.

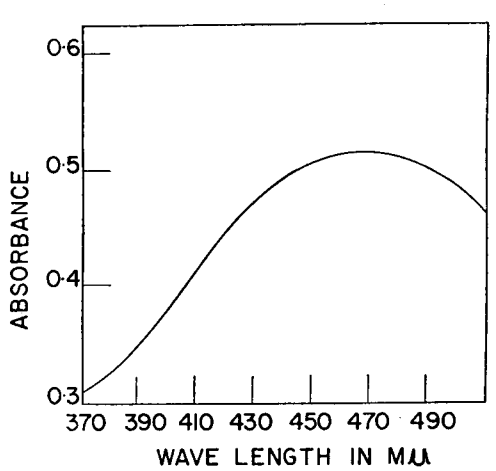

Figure 1. Absorption spectrum of solution of *N,N*-diethylnicotinamide–decaborane adduct in cyclohexane using 100 γ of decaborane per cc.

Beckman DU spectrophotometer, 1-cm. absorption path length

PROCEDURE

Five cubic centimeters of a 25% aqueous solution of *N,N*-diethylnicotinamide were added to 0.5 cc. of sample containing decaborane. The color development became stabilized in 90 minutes at room temperature. Readings were taken at 435 mμ using a Beckman DU spectrophotometer. The color reagent may be obtained in this dilution from a number of supply houses under the name of Nikethamide.

RESULTS AND DISCUSSION

Solutions of decaborane in cyclohexane were used to determine the absorption curve of the *N,N*-diethylnicotinamide–decaborane adduct. As shown in Figure 1, a broad region of maximum absorption was obtained between 430 and 500 mμ.

The decaborane used for the standardization curve was purified by vacuum sublimation. From a stock solution containing 50 mg. of decaborane in 100 cc. of cyclohexane, dilutions in this solvent were prepared to provide a range of 5 to 200 γ per 0.5 cc. of sample used for analysis. Samples containing 10 and 120 γ of decaborane gave absorbance values of 0.05 and 0.585, respectively. Within these limits, using five sample concentrations, the concentration of decaborane and the absorbance were related linearly.

The nature of *N,N*-diethylnicotinamide–decaborane adduct is not known with certainty. Some information of its structure was obtained by comparing its infrared absorption spectrum as a film with that of a film of decaborane as recorded with a Perkin-Elmer single-beam infrared spectrometer, Model 12 C. Using the frequency assignments determined by Keller and Johnston (*2*), it appeared that reaction occurred at the B-H . . . B bridge structure of the decaborane molecule, since the absorption bands due to these structures were no longer found in the adduct. Boron compounds not having the bridge structure, such as boric acid and a variety of boron salts, were found to produce no color with the reagent.

Although decaborane is insoluble in water, substances such as 1% gelatin or undiluted blood plasma may form stable dispersions of decaborane in low concentrations—e.g., 1 mg. per cc. Such dispersions, immediately after preparation, gave readings with the color reagent, corrected for blank values, essentially the same as with equal concentrations of decaborane in cyclohexane. The presence of the proteins did not interfere with color development in the reaction.

The color reaction with diborane and pentaborane was examined also. No color was obtained with diborane in the conditions of analysis, probably because of the known (5) rapid rate of hydrolysis of diborane in contact with water. With pentaborane a transitory red color was obtained which almost immediately changed to yellow. Absorption between 430 and 500 mμ was negligible in the concentration range used for decaborane. Of these boranes the method thus seems restricted to decaborane.

The reaction of decaborane with *N,N*-diethylnicotinamide appears to be fundamentally similar to that reported by Hill and Johnston (1) using quinoline. It has an additional advantage to the biochemist, over the quinoline method, in that it is directly applicable to aqueous solutions.

LITERATURE CITED

(1) Hill, W. H., and Johnston, M. S., ANAL. CHEM. **28**, 1300 (1955).
(2) Keller, W. E., and Johnston, H. L., *J. Chem. Phys.* **20**, 1749 (1952).
(3) Schlesinger, H. I., and Burg, A. B., *Chem. Revs.* **31**, 1 (1942).
(4) Stock, A., "Hydrides of Boron and Silicon," Cornell University Press, Ithaca, N. Y., 1933.
(5) Stock, A., and Kuss, E., *Ber.* **47**, 810 (1914).

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Quantitative Determination of Ethylene Glycol in Water

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A rapid and accurate procedure suitable for large numbers of determinations of ethylene glycol in water has been developed. It is especially useful where greater accuracy than a hydrometer determination is necessary. This procedure can be applied to the determination of glycerol, any vicinal glycol or ketone, α-hydroxyaldehyde, ketone, or acid in water, as long as the reaction rates of each substance are within practical limits.

A RAPID and accurate procedure which utilizes only standard laboratory equipment has been developed for quantitatively determining ethylene glycol in water. The procedure is based on the well-known periodate scission of vicinal glycols originally proposed by Malaprade (2), followed by formation of the insoluble silver iodate.

The qualitative procedure for the determination of glycols in alkyd resins (1) involves the standard reaction, which may be written as follows:

$$\begin{matrix} R\,CHOH \\ | \\ R\,CHOH \end{matrix} + HIO_4 \xrightarrow{HNO_3} 2RCHO + HIO_3 + H_2O$$

$$HIO_3 + AgNO_3 \longrightarrow \underset{\text{white precipitate}}{\overset{AgIO_3}{\downarrow}} + HNO_3$$

The test depends on the fact that silver iodate is nearly insoluble in dilute nitric acid, whereas silver periodate, if formed as such, is soluble. During the investigation of glycol in alkyd resin, it was noted that the amount of nitric acid added in the final glycol determination in the procedure was critical, and that, if the glycol concentrations were varied and the concentration of the acid was held constant, the length of time it took the precipitate to form varied, and this time could be accurately reproduced for each glycol concentration. By making use of this information, it was found possible to determine any percentage of ethylene glycol from 0.10 to 100% by standardizing the nitric acid concentration in the test and recording the exact length of time it took for the silver iodate precipitate to appear.

PROCEDURE

Place 2.00 ± 0.01 ml. of the ethylene glycol sample to be tested in a test tube (22 × 175 mm.). Add 2.0 ml. of 0.1*N* aqueous silver nitrate. Pipet into the test tube 5.0 ml. of an acid solution containing 80 ml. of concentrated nitric acid and 4.56 grams of periodic acid ($HIO_4 \cdot 2H_2O$) per liter of solution. Shake thoroughly. By means of an accurate timer, record the length of time which expires from the moment the first drop of acid mixture reaches the sample until the white precipitate appears. A black background will aid in recognizing the appearance of this precipitate. If the precipitate forms in less than 23.5 seconds, dilute a portion of the sample tenfold by volume and repeat the test on this mixture. If the precipitate still forms in less than 23.5 seconds, dilute another portion of the sample one hundredfold by volume and repeat the test on this mixture. In order to obtain maximum accuracy, the mixture tested should contain from 0.10 to 1.00% glycol. One of the above dilution ratios will insure this range.

NOMOGRAPH

A nomograph has been constructed to facilitate the calculation of glycol percentages. The data obtained on the mixtures containing 0.10 to 1.00% glycol, as shown in Table I, are plotted as

Table I. Data Establishing Relationship between Ethylene Glycol Concentration and Reaction Time

Ethylene Glycol, Weight %	Reaction Time, Seconds (Average of 5 to 10 Runs)
0.0998	135
0.1995	81
0.2993	60
0.3990	48
0.4988	39
0.5986	34
0.6983	30
0.7981	27
0.8978	25
0.9976	23.5

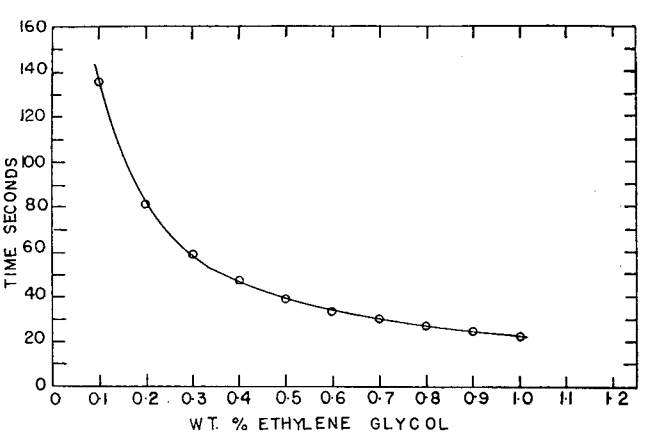

Figure 1. Plot of reaction time *vs.* per cent of ethylene glycol by weight

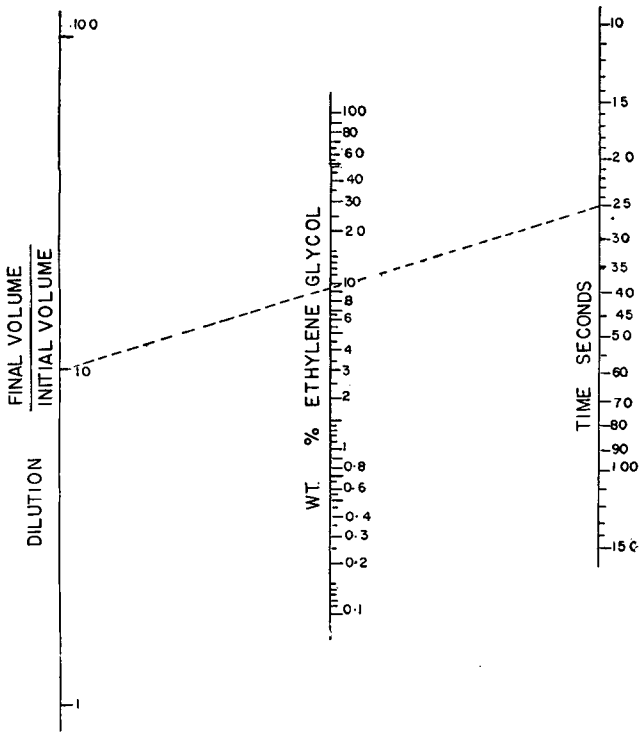

Figure 2. Nomograph for rapid computation of per cent of ethylene glycol from observed reaction time

shown in Figure 1. Using this graph, the empirical equation obtained from this data is $G = \frac{63.10D}{T^{1.31}}$, where G is the per cent of ethylene glycol by weight, D is the dilution ratio by weight, and T is the time of reaction in seconds. The nomograph shown in Figure 2 was constructed from this equation. A sample solution is shown in Figure 2, wherein a 10 to 1 dilution required 25 seconds; the concentration of the original sample is then read from the middle scale as 9.5% of ethylene glycol by weight.

The procedure as set up requires a volumetric dilution of 100 to 1 for concentrations above 10% glycol. Because of the difference in density between ethylene glycol and water, an error is introduced by the volumetric dilution of solutions. In less than 10% solutions, this error is not significant; however, in the higher concentrations this error becomes appreciable. The mark for a 100 to 1 dilution on the nomograph has therefore been modified to correct for a 50% solution, thus minimizing the error introduced by volumetric dilution. Theoretically, the most accurate procedure would involve dilution by weight. However, the time required to make such a dilution would detract from the usefulness of the procedure.

DISCUSSION

Tables II and III are representative of the reproducibility and accuracy that may be obtained by the method. As can be seen in Table III, the maximum error based on ethylene glycol present is 1.083% and this error is found with the most concentrated glycol solution. This makes the procedure especially useful when results on large numbers of samples are needed and when greater accuracy is needed than is given by a hydrometer. Accuracy is dependent upon laboratory technique. If a nomograph is used to read the results, a 30-inch scale for the per cent ethylene glycol is recommended.

Any substance which reduces the periodate radical to the iodate radical, or which gives a precipitate with silver nitrate in acid solution, interferes with the procedure.

Table II. Data Illustrating Reproducibility of Method

Ethylene Glycol, Weight %	Dilution Ratio (by Volume)	Representative Reaction Times, Sec.
0.0998	1:1	130.0 139.3 140.4 135.8
0.2495	1:1	68.0 67.4 69.2 68.6
0.4988	1:1	38.9 39.3 39.3 39.2
0.9976	1:1	23.5 23.5 23.7 23.5
4.988	10:1	39.6 40.0 38.9 39.5
9.976	10:1	23.7 23.5 23.7 23.4
24.950	100:1	67.5 68.2 69.0 68.0
49.880	100:1	38.4 38.2 38.4 38.5
74.850	100:1	27.6 27.7 27.8 28.0
99.769	100:1	22.2 22.2 22.1 22.1

Table III. Comparison of Known Per Cents of Ethylene Glycol with Values Computed from Observed Reaction Time

Known Ethylene Glycol, Weight %	Average Reaction Time, Sec.	Value[a] of $63.10D$	Computed % Ethylene Glycol Using Equation $\%G = 63.10D/T^{1.31}$	Error
0.0998	135.0	63.10	0.1020	+0.0022
0.2495	68.3	63.10	0.2490	−0.0005
0.4988	39.2	63.10	0.5150	+0.0162
0.9976	23.5	63.10	1.007	+0.0031
4.988	39.5	627.0	5.070	+0.082
9.976	23.8	624.0	9.815	−0.161
24.950	68.2	6174	24.456	−0.494
49.880	38.4	5985	50.300	+0.420
74.850	27.8	5834	74.865	+0.015
99.760	22.2	5727	98.677	−1.083

[a] In conducting tests for this table, this value was obtained by weighing initial and final volume of mixtures in order to obtain dilution ratio by weight, and multiplying this ratio by constant, 63.10.

The procedure can be applied to the determination of glycerol, any vicinal glycol, α-hydroxyaldehyde, α-hydroxyketone, vicinal ketone, or α-hydroxy acid in water, as long as the reaction rates of each substance are within practical limits.

Ethylene glycol, C. P., was used as received from Eimer and Amend, and contained 0.24% moisture determined by the Karl Fischer method.

All solutions were prepared and tests were conducted at 73° F. As can be expected, temperature variations affect the reaction rate. The procedure may be used at other constant temperatures, provided different sets of constants are determined for the basic equation for each temperature.

LITERATURE CITED

(1) Jordan, C. B., ANAL. CHEM. 26, 1657 (1954).
(2) Malaprade, L., *Compt. rend.* 186, 382 (1928).

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Spectrophotometric Titration of Milligram Quantities of Barium

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The detection of the end point is difficult in the complexometric titration of small amounts of barium with disodium dihydrogen ethylenediaminetetraacetic acid using Eriochrome Black T as indicator. A method is described for the determination of barium which utilizes a spectrophotometric detection of the end point. The method is capable of determining 0.1 to 5 mg. of barium by titration with 0.01 to 0.001*N* EDTA at a wave length of 650 mμ.

MANNS, Reschovsky, and Certa (*3*) have published a titrimetric method for barium in which 500 mg. of barium acetate was titrated with 0.1*N* disodium dihydrogen ethylenediaminetetraacetic acid [disodium dihydrogen(ethylenedinitrilo)tetraacetic acid, EDTA]. The concentration of dye used by these investigators was such that under tungsten illumination a color change from wine-red to almost colorless was observed at the end point. The application of this procedure by others (*4*, *5*) to smaller amounts of barium has indicated the use of more dilute titrant and additional dye in order to detect the end point. Under these conditions the color change is extremely difficult to detect, as the solution is titrated until the last trace of wine-red color disappears leaving a clear blue color. The elimination of this visual difficulty by utilizing a spectrophotometric detection of the end point is described in the present paper. In addition, this technique permits the determination of much smaller quantities of barium than is possible with the visual method.

MATERIALS USED

EDTA solutions. A 0.01*N* solution was prepared according to the directions of Manns, Reschovsky, and Certa (*3*), except for appropriate reductions in the quantities of reagents required. This solution was standardized by the titrimetric procedure given below against a barium solution which had been previously standardized by a gravimetric procedure. A 0.002*N* EDTA solution was prepared from the 0.01*N* solution by dilution.

The buffer solution was also prepared according to directions of Manns (*3*).

Eriochrome Black T. Fifty milligrams of the dye (Hartman-Leddon Co., Philadelphia, Pa.) were dissolved in 50 ml. of triethanolamine (*1*).

Titration apparatus. A Coleman Universal instrument, Model 14, was used with a rectangular titration cell of 75-ml. capacity with a 5-cm. light path. A small propeller-type glass stirrer and a bent glass tube which hooked on to the cell and could be used to furnish a blanket of nitrogen to exclude carbon dioxide completed the cell assembly.

PROCEDURE

Take for analysis a sample which contains from 0.1 to 5 mg. of barium in 15 to 20 ml. of solution which should be neutral or slightly acid and carbonate-free. Add in the following order: 5 ml. of methanol, 10 ml. of buffer, and 5 drops of the indicator dye to the titration cell. Adjust the stirrer so that it is above the light path and so that the solution is agitated without the appearance of air bubbles; make sure that the solution contents are thoroughly mixed before the titration is begun. Titrate with a standard EDTA solution measuring the absorbancy at 650 mμ. Use 0.002*N* EDTA solution for the titration of less than 1 mg. of barium; for larger amounts use 0.01*N* titrant. Obtain the end point from a graph of milliliters versus absorbancy. Determine a reagent blank correction from a similar titration in the absence of barium.

RESULTS AND DISCUSSION

The maximum difference between the absorbancy of the magnesium complex with Eriochrome Black T dye and the dye itself was found to occur at 650 mμ. This is in agreement with data by Harvey, Komarmy, and Wyatt (*2*).

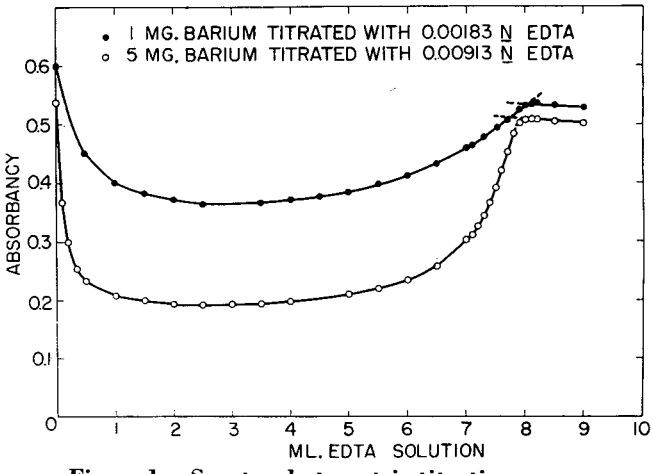


Figure 1. Spectrophotometric titration curves

Table I. Determination of Barium

Ba^{++} Taken, Mg.	Difference (Found Minus Taken), Mg.
0.10	0.00, 0.00, 0.00
0.49	+0.01, 0.00, 0.00, −0.02
0.99	−0.01, −0.01, −0.01, −0.01, −0.01
4.94[a]	0.00, +0.04, +0.06, +0.03

[a] Titrated with 0.00913*N* EDTA; all other titrations with 0.00183*N* EDTA.

Data obtained at this wave length in the titration of milligram quantities of barium are shown in Table I. Typical titration curves are shown in Figure 1. The absorbancy readings were not corrected for volume changes in the solution.

A reagent blank correction of 0.14 ml. was deducted when the 0.00183*N* EDTA solution was used. The blank was negligible in the case of the 0.00913*N* solution.

Several titrations with 50-mg. quantities of barium and 0.1*N* EDTA indicate the feasibility of determining larger amounts of barium; in four titrations the volumes of titrant were 6.98, 6.98, 7.03, and 7.06 ml. In the titration of this large quantity of barium it is necessary to blanket the solution with nitrogen to prevent the precipitation of barium carbonate.

ACKNOWLEDGMENT

One of the authors (L. G.) gratefully acknowledges the support of the Atomic Energy Commission under Contract AT(30-1)-1213 with Syracuse University.

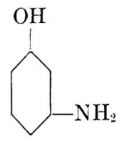
LITERATURE CITED

(1) Diskant, E. M., ANAL. CHEM. 24, 1857 (1952).
(2) Harvey, A. E., Komarmy, J. M., and Wyatt, G. M., *Ibid.*, 25, 498 (1953).
(3) Manns, T. J., Reschovsky, M. U., and Certa, A. J., *Ibid.*, 24, 909 (1952).
(4) Reimer, C., doctoral thesis, Syracuse University, 1953.
(5) Sijderius, R., *Anal. Chim. Acta* 10, 517 (1954).

RECEIVED for review December 30, 1954. Accepted October 26, 1955. Work carried out under the auspices of the U. S. Atomic Energy Commission.

102. *m*-Aminophenol (3-Aminophenol)

Contributed by JOHN KRC, JR., and RALPH HINCH, JR., Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.



Structural Formula for *m*-Aminophenol

DURING the previous crystal studies on 4-aminosalicylic acid (*2*) a decomposition product was observed. This compound, not identified at that time, was obtained virtually pure when 4-aminosalicylic acid was sublimed or melted, and has now been identified as *m*-aminophenol and characterized.

Excellent crystals of *m*-aminophenol can be obtained by vacuum sublimation of *p*-aminosalicylic acid or by direct sublimation of the aminophenol itself.

CRYSTAL MORPHOLOGY

Crystal System. Orthorhombic.

Form and Habit. Needles and rods elongated parallel to *a* lying on brachypinacoid, {010}, or basal pinacoid, {001}, showing brachydome, {011}, and the prism {120}.

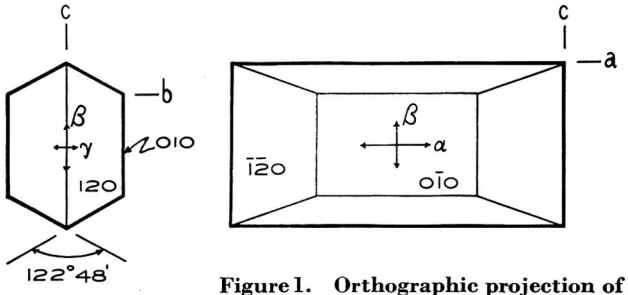
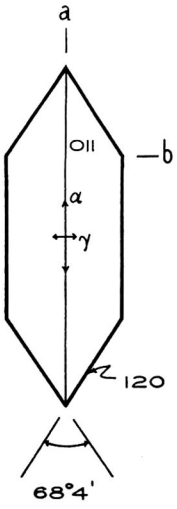


Figure 1. Orthographic projection of typical crystal of *m*-aminophenol



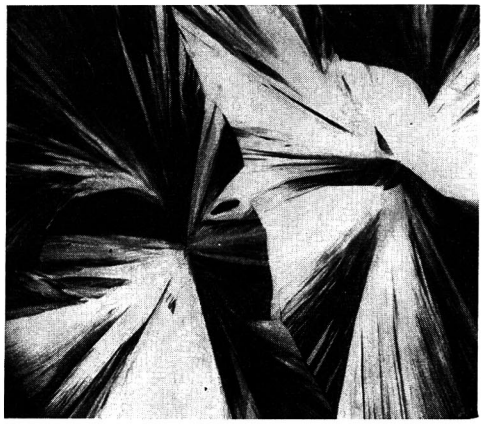


Figure 2. Crystals of *m*-aminophenol from melt

Axial Ratio. $a:b:c = 0.740:1:0.545$.

Interfacial Angles (Polar). $120 \wedge \bar{1}20 = 111° 56'$; $120 \wedge 010 = 34° 2'$; $011 \wedge 01\bar{1} = 122° 48'$; $011 \wedge 001 = 28°36'$; $011 \wedge 010 = 61° 24'$.

Cleavage. Excellent, parallel to 100 and 010.

X-RAY DIFFRACTION DATA

Space Group. $C_{2v}^2(Pmc\ 2_1)$ (*1*).

Cell Dimensions. $a = 8.31$ A.; $b = 11.23$ A.; $c = 6.12$ A. $a = 6.14$ A.; $b = 11.10$ A.; $c = 8.38$ A. (*1*).

Formula Weights per Cell. 4 (4.02 calculated from x-ray data).

Formula Weight. 109.12.

Density. 1.275 (flotation in carbon disulfide–carbon tetrachloride; pycnometer); 1.269 (x-ray).

Principal Lines

d	*I*/*I*$_1$	*d*	*I*/*I*$_1$
5.37	8	2.40	6
4.66	6	2.33	Weak
4.51	10	2.29	5
4.14	9	2.17	3
3.72	9	2.11	Weak
3.42	7	2.08	Weak
3.33	8	2.04	2
3.28	8	2.00	Weak
3.18	4	1.924	2
2.92	6	1.870	Weak
2.77	4	1.848	1
2.65	Weak	1.823	1
2.54	2	1.801	1
2.47	4	1.791	1
2.45	4	1.767	2

OPTICAL PROPERTIES

Refractive Indices (5893 A.; 25° C.). $\alpha = 1.583 \pm 0.002$. $\beta = 1.666 \pm 0.002$. $\gamma = 1.775 \pm 0.005$.

Optic Axial Angles (5893 A.; 25° C.). $2H = 97°$. $2V = 87°$ (calculated from β and $2H$); $2V = 87°$ (calculated from α, β, and γ).

Dispersion. $r > v$, very weak.

Optic Axial Plane. 001.

Sign of Double Refraction. Positive.

Acute Bisectrix. $\gamma = b$.

Molecular Refraction (R) (5893 A.; 25° C.). $\sqrt[3]{\alpha\beta\gamma} = 1.673$. R(calcd.) = 32.14; R (obsd.) = 32.05.

FUSION DATA. *m*-Aminophenol melts at 122° C. with needle-like sublimate but no decomposition. The melt supercools readily but crystallizes even after cooling to room temperature to give rounded plates lying on 100; these soon develop into slowly growing spherulites. The 100 plates show a nearly centered B_{X_0} figure with $2V = (+)87°$, weak dispersion ($r > v$). On a melt-back the melt crystallizes as blades having a well-formed profile angle of about 80°. There is no indication of polymorphism.

ACKNOWLEDGMENT

The assistance of Barbara Wehringer in purifying this compound and of Muriel Clark in determining the x-ray powder data is gratefully acknowledged. This work was carried out in cooperation with the Department of Pharmacology, University of Chicago, and was supported in part by funds from the Illinois Tuberculosis association.

LITERATURE CITED

(1) Caspari, W. A., *Phil. Mag.* **4**, 1276–85 (1927).
(2) Krc, J., Jr., and McCrone, W. C., ANAL. CHEM. **27**, 1503 (1955).

CONTRIBUTIONS of crystallographic data for this section should be sent to Walter C. McCrone, Analytical Research, Armour Research Foundation of Illinois Institute of Technology, Chicago 16, Ill.

The "Separation Factor"

SIR: In the March 1954 issue of ANALYTICAL CHEMISTRY there appear three papers (*12–14*) by Boyd Weaver, in which he introduces the "separation factor" as a criterion for the evaluation of fractional separation processes. The undersigned believe that the general application of the separation factor, as proposed by Weaver, can lead to completely erroneous conclusions.

First, one might infer from these papers that the term "separation factor" is new so far as precipitation processes are concerned. However, Weaver's equation (*12*) utilizing this factor is mathematically identical with that proposed by Henderson and Kracek (*6*)

$$D = \frac{(\mathrm{Ra/Ba})_{\mathrm{crystals}}}{(\mathrm{Ra/Ba})_{\mathrm{solution}}}$$

It should be noted for the record that Henderson and Kracek were criticized by Chlopin (*2*) because the latter author (*1*) had previously stated an identical expression, as follows:

$$K_f = \frac{\text{in den Kristallen enthaltendes Ra in Prozenten der Gesamt-Ra-Menge}}{\text{in den Kristallen enthaltendes Ba in Prozenten der Gesamt-Ba-Menge}} \Big/ \frac{\text{in d. Mutterlauge verbliebenes Ra in Prozenten d. Gesamt-Ra-Menge}}{\text{in d. Mutterlauge verbliebenes Ba in Prozenten d. Gasamt-Ba-Menge}}$$

As another example, we cite the text by Kolthoff and Sandell (*8*) which shows the following equation:

$$\frac{(\mathrm{Ba})_{\mathrm{solution}}}{(\mathrm{Ba})_{\mathrm{precipitate}}} = D\,\frac{(\mathrm{Pb})_{\mathrm{solution}}}{(\mathrm{Pb})_{\mathrm{precipitate}}}$$

Secondly, and more important, Weaver apparently overlooks another aspect of precipitation processes. This is concerned with the mode of distribution within the crystal of a substance coprecipitated with another as in a fractional precipitation process. The separation factor—commonly known as the homogeneous distribution coefficient (*11*)—is valid for a system in which one component is homogeneously distributed throughout the precipitate. In another mode of distribution, one component is logarithmically distributed. Doerner and Hoskins (*3*) describe such a system by the following equation:

$$\log \frac{\mathrm{Ra}^{++}_{\mathrm{initial}}}{\mathrm{Ra}^{++}_{\mathrm{final}}} = \lambda \log \frac{\mathrm{Ba}^{++}_{\mathrm{initial}}}{\mathrm{Ba}^{++}_{\mathrm{final}}}$$

The logarithmic distribution coefficient, λ, is valid for the case where equilibrium exists at all times between each infinitesimal layer of the crystal, during the process of its formation, and the solution. This is in contrast with the homogeneous distribution concept which demands that the whole crystal must at all times be in equilibrium with its solution.

Systems, such as Weaver's (*13, 14*), which utilize precipitation from homogeneous solution, would normally be characterized by a logarithmic distribution because of the gradual formation of the precipitate and the concomitant equilibrium state between the successive crystal surfaces and the solution (*3, 5, 7, 9, 10*). On the other hand, a long contact time between crystal and solution, with possible resulting resolution, might partially disrupt the system so that it is characterized by neither mode of distribution. We believe that this is the case with the data of Table II of the third Weaver paper (*14*), as is shown by the first four values in Table I.

One can conclude from Table I that because the four values for D are no more constant than those for λ, the homogeneous distribution equation used by Weaver is no more valid for his oxalate system than is the logarithmic distribution equation.

All the data of Table I were included by Weaver in a previous report (*15*), with a conclusion that there is apparently no correlation between separation factor and degree of precipitation. However, in the subsequent publication (*14*) in ANALYTICAL CHEMISTRY, he concluded, on the basis of only part of the data of Table I, that there appeared to be a slight trend for the separation to improve as the degree of precipitation is increased; with respect to this latter statement, it is possible to select data from (*15*) for an opposite conclusion. The undersigned do not understand why any of the data were discarded in order to arrive at a different conclusion. In either case, a trend in the separation factor indicates that the homogeneous distribution law is not completely valid for this system.

In two of Weaver's papers (*12, 13*) separation factors have been calculated for a praseodymium-cerium mixture from data by Gordon, Brandt, Quill, and Salutsky (*4*). The latter paper contains no data for praseodymium-cerium mixtures, but only data for praseodymium-lanthanum and cerium-lanthanum mixtures. We wonder, therefore, how these data were used to calculate separation factors for praseodymium-cerium mixtures.

Table I. Fractionation of Samarium-Neodymium Mixtures

Degree of Precipitation, %	Separation Factors, D	λ[a]
16.8[b]	1.64[b]	1.59
36.3[b]	1.66[b]	1.52
55.7[b]	1.70[b]	1.44
75.1[b]	1.83[b]	1.38
2.8	1.56	
6.5	1.46	
7.0	1.74	
16.2	1.55	
35.7	1.63	
44.8	1.66	
45.1	1.78	
52.4	1.67	
54.2	2.14	
74.3	1.54	
83.5	2.12	
89.2	1.42	

[a] λ values calculated by us. Remaining data taken from ORNL-1629 (*15*).
[b] Data by Weaver (*14*).

In the paper describing the use of mandelic acid (*13*), separation factors are reported in Tables V, VI, and VII for pairs of rare earths in complex systems containing from four to seven rare earths. It is our belief that it may be incorrect to apply a distribution law describing a binary system to such a complicated mixture of rare earths. When we calculated separation factors for those instances where there are blank spaces in the tables, we obtained several values less than 1.0. In these cases the order of precipitation of the elements would be the reverse of that reported by Weaver.

LITERATURE CITED

(1) Chlopin, V., *Z. anal. allgem. Chem.* **143**, 97 (1925).
(2) Chlopin, V., *Z. anorg. Chem.* **172**, 318 (1928).
(3) Doerner, H. A., and Hoskins, W. M., *J. Am. Chem. Soc.* **47**, 622 (1925).
(4) Gordon, L., Brandt, R. A., Quill, L. L., and Salutsky, M. L., ANAL. CHEM. **23**, 1811 (1951).
(5) Gordon, L., Reimer, C. C., and Burtt, B. P., *Ibid.*, **26**, 842 (1954).

(6) Henderson, L. M., and Kracek, F. C., *J. Am. Chem. Soc.* **49**, 738 (1927).
(7) Hermann, J. A., "Separation of Americium from Lanthanum by Fractional Oxalate Precipitation from Homogeneous Solution," U. S. Atomic Energy Commission, **AECD-3637** (July 22, 1954).
(8) Kolthoff, I. M., and Sandell, E. B., "Textbook of Quantitative Inorganic Analysis," 3rd ed., p. 123, Macmillan, New York, 1952.
(9) Salutsky, M. L., Stites, J. G., and Martin, A. W., ANAL CHEM. **25**, 1677 (1953).
(10) Shaver, K., Division of Physical and Inorganic Chemistry, 125th Meeting, AM. CHEM. SOC., Kansas City, Mo., 1954.
(11) Wahl, A. C., and Bonner, N. A., "Radioactivity Applied to Chemistry," p. 106, Wiley, New York, 1951.
(12) Weaver, B., ANAL. CHEM. **26**, 474 (1954).
(13) *Ibid.*, p. 476.
(14) *Ibid.*, p. 479.
(15) Weaver, B., "Fractional Separation of Rare Earths by Oxalate Precipitation from Homogeneous Solution," U. S. Atomic Energy Commission, **ORNL-1629** (Dec. 4, 1953).

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SIR: In an article published in ANALYTICAL CHEMISTRY [**26**, 474 (1954)] I pointed out that most workers engaged in difficult separations, such as between the rare earths, have failed to publish any standards by which the efficiency of their separations can be measured.

In my own separations, and I assumed in those of most other workers, there was a schematic arrangement of pairs of fractions. The two fractions of a pair were usually of nearly equal quantity. As I was interested in the difference between the members of a pair, I expressed the degree of separation of two elements as the ratio of the relative abundances of these elements in the two fractions and called this the separation factor. I applied this relationship to the measurement of the efficiency of the few other separations published with adequate data, and in two concurrently published articles applied it to my own work on two methods of separating rare earths.

Subsequently L. Gordon and M. L. Salutsky have pointed out that this homogeneous distribution factor, D, applies strictly only to separations in which the two fractions are in complete equilibrium throughout the process of separation, as in liquid-liquid extractions. In precipitations or crystallizations, especially those made from homogeneous solutions, the solution is in equilibrium only with the surfaces of the solids. The separation efficiency here can be measured more accurately by the logarithmic distribution coefficient,

$$\lambda = \log \frac{A_{\text{initial}}}{A_{\text{final}}} / \log \frac{B_{\text{initial}}}{B_{\text{final}}}$$

Both factors have appeared in previous publications. The applicability of each expression can be tested by calculating it for different degrees of precipitation.

In my article on separation of rare earths by oxalate precipitation I applied the D factor to a few varying degrees of precipitation of samarium-neodymium mixtures consisting initially of equal amounts of the oxides of these elements. Subsequently the data were extended by more experiments. I have now calculated both D and λ factors for the full set of experiments. Attention must be called to the fact that when the extent of precipitation is small, the composition of the final fraction is only slightly different from that of the initial material. A slight error in analysis, easily made in the case of rare earths, can make a great difference in the logarithmic ratio. In the present case the compositions of the final fractions have been calculated from those of the original mixtures and the precipitates, as there appeared to be less error in these analyses. This minimizes the error in λ factors for small degrees of precipitation, but leaves considerable possibility of error in extensive precipitations, where the compositions of the total precipitate and original are similar.

Table I. Fractionation of Samarium-Neodymium Mixtures

(Variation in degree of precipitation)

Degree of Pptn., %	Ratios of Sm_2O_3 to Nd_2O_3[a]		Separation Factor	
	Precipitate	Filtrate (calcd.)	D	λ
2.8	1.59	0.988	1.61	1.55
6.5	1.49	0.973	1.53	1.52
7.0	1.68	0.962	1.75	1.73
16.2	1.44	0.931	1.55	1.51
16.8	1.66	0.904	1.84	1.75
35.7	1.35	0.848	1.59	1.22
36.2	1.48	0.800	1.85	1.65
44.8	1.33	0.792	1.68	1.48
45.1	1.35	0.782	1.73	1.51
52.4	1.32	0.737	1.79	1.51
54.2	1.30	0.735	1.77	1.50
55.7	1.33	0.700	1.90	1.55
74.3	1.24	0.530	2.34	1.50
75.1	1.22	0.537	2.27	1.55
83.5	1.13	0.528	2.14	1.42
89.2	1.03	0.770	1.34	1.12

[a] Originally 1:1.

The data in Table I show the λ factor to be considerably more nearly constant than the D factor. However, its use does leave one without a simple relationship between the products of a separation.

I do not know why the λ values calculated solely on the basis of analyses of precipitates are more nearly constant than if calculated from filtrates, but this is a fact in this instance. As D values involve both fractions, they are not so greatly affected by analytical inaccuracies in only one fraction.

I can understand the critics' puzzlement regarding the difference in ORNL-1629 and the published article. The embarrassing fact is that, while the article was not submitted until after ORNL-1629 had been written, the copy submitted was an earlier version written some time before the additional work given in the larger table had been done. There was no selection of items. The enlarged table had been given at Chicago, and I intended to publish it.

While all analyses of rare earths are subject to some error, the most obviously inaccurate analyses were obtained in the case of 16.8% precipitation, the case which Gordon and Salutsky continually point out as typical example. I should prefer to omit it from the table.

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"Standard Addition" Method of Polarographic Analysis

SIR: One of the most valuable techniques of quantitative polarographic analysis is the standard addition method devised by Hohn (*1*) and discussed by Kolthoff and Lingane (*2*), Taylor (*4*), and Meites (*3*). Starting with a known volume of the sample, the diffusion current, i_1, of the desired wave is measured; then a known volume of a known solution of the substance being determined is added, and the diffusion current, i_2, is measured again. The calculation of the concentration of the substance in question in the original solution follows from an equation of the form

$$C_u = i_1 v C_S / [i_2(V + v) - i_1 V] \quad (1)$$

where C_u and C_S are the concentrations of the sample and the standard, respectively, and V and v are the corresponding volumes.

Reiterated in the literature on this simple technique are statements to the effect that for maximum precision the amount of standard solution added should be sufficient just about to double the wave height. As this would be a nuisance in practical work, and the truth of this assertion seems to have been generally accepted, the present demonstration of its falsity would appear to be of some interest.

It is convenient to assume that the error in C_u arises from errors in i_1 and i_2 alone—in other words, that v, V, and C_S are measured with considerably better accuracy than the two diffusion currents. It is also convenient to assume that, as is ordinarily true in practice, V is much larger than v, although the validity of the conclusion is in no way affected if this is not the case. This permits rewriting Equation 1 in the form

$$C_u = k\, i_1/(i_2 - i_1)$$

A simple differentiation then gives the relative error of the result:

$$\frac{dC_u}{C_u} = \frac{di_1}{i_1} + \frac{di_1}{i_2 - i_1} - \frac{di_2}{i_2 - i_1} \qquad (2)$$

The first term on the right of Equation 2 is simply the relative error of i_1. For a given relative error of measurement of i_1 or i_2, however, the second and third terms have their smallest values, not at $i_2 = 2i_1$, but at $i_2 >> i_1$. In other words, maximum precision is attained when the diffusion current of the unknown is negligibly small compared to that obtained after adding the standard.

If it is assumed that a 1% error may be made in each diffusion current measurement, the relative error of the answer may reach 4% if $i_2 = 2i_1$. If $i_2 = 10i_1$, however, the corresponding relative error of the answer cannot exceed 2.2%, which is almost a twofold gain in accuracy.

It is evident from this simple analysis that the amount of standard solution added should actually be sufficient to increase the observed diffusion current by a factor of 10 or so, except in the event that the diffusion current is a linear function of concentration over too narrow a range to permit so large an addition.

LITERATURE CITED

(1) Hohn, H., "Chemische Analysen mit dem Polarographen," p. 51, Springer-Verlag, Berlin, 1937.
(2) Kolthoff, I. M., and Lingane, J. J., "Polarography," p. 251, Interscience, New York, 1941.
(3) Meites, L., "Polarographic Techniques," p. 178, Interscience, New York, 1955.
(4) Taylor, J. K., ANAL. CHEM. 19, 368 (1947).

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MEETING REPORT

Society for Analytical Chemistry

A MEETING of the Scottish Section was held Sept. 30 at Glasgow, at which the following papers were presented and discussed.

Lead in Biological Materials. S. L. TOMPSETT, Northern General Hospital, Edinburgh.

The clinical applications were described under the headings: the level of blood lead as an aid in the diagnosis and treatment of lead poisoning in the human, the excretion of lead, and the distribution of lead in the tissues of the "normal" subject and in cases of lead poisoning. The determination of lead in urine, feces, blood, soft tissues, and bone was then dealt with under the headings: destruction of organic matter, separation of lead with ether as a diethyldithiocarbamate complex, and colorimetric determination with dithizone by the reversion technique.

Determination of Lead by Square-wave Polarography. D. J. FERRETT, A.E.R.E., Harwell, Berks.

A brief survey of the principles of square-wave polarography illustrates how, with this technique, reducible metal ions—e.g., lead—may be determined at concentrations down to 2×10^{-7} (0.05 γ of lead per ml.). The differential nature of the technique eliminates much of the chemical treatment normally needed before polarographic determinations. These advantages were illustrated in the determination of lead in cocoa (0.7 p.p.m.), in analytical reagents (0.7 p.p.m.), in aluminum, zinc, and tin-base alloys (0.003 to 0.1%), and in steels (0.2%).

At a meeting on Oct. 5 in London, the following papers were presented.

Colorimetric Determination of Phosphorus in Steel and Copper-Base Alloys. W. T. ELWELL AND H. N. WILSON, Physical Chemistry Group, I.C.I., Ltd., Billingham.

A colorimetric procedure was recommended for the determination of phosphorus in all classes of steel; it has also been successfully applied to two standard bronze samples. The procedure permits a considerable saving in time, particularly in the examination of highly alloyed steels, when the determination can be completed in under 1.5 hours, compared with about 8 hours required by the existing gravimetric method.

The determination is based on the formation of phosphovanadomolybdic acid, which is soluble in amyl alcohol. The yellow color, which is proportional to the amount of phosphorus present, does not fade and can be measured in any convenient way.

Determination of Small Amounts of Carbon in Steel by Low-Pressure Analysis. R. M. COOK AND G. E. SPEIGHT, Mond Nickel Co., Ltd., Birmingham, and Richard Thomas & Baldwin, Bucks.

A simplified low-pressure method was described for the determination of carbon in steel. The use of a normal combustion furnace in conjunction with the low-pressure analytical apparatus permits samples to be analyzed with a precision of ±0.0001% of carbon up to 0.036% of carbon, the deviation being slightly greater with higher carbon contents.

The method lends itself readily to the analysis of carbon contents on a semimicro basis when only small weights of sample are available. With medium and high carbon steels, analyses may be carried out on samples weighing as little as 0.05 gram with a degree of accuracy at least equal to that given by the normal gravimetric combustion method operated under the best conditions, 2.729-gram samples being used.

Determination of Small Amounts of Sulfate by Reduction of Hydrogen Sulfide, and Titration with Mercuric or Cadmium Salts using Dithizone as Indicator. E. E. ARCHER, Distillers Co., Ltd., Great Burgh, Epsom, Surrey.

Sulfate is reduced to hydrogen sulfide with an acid reduction mixture. The hydrogen sulfide is absorbed in an alkaline solution containing dithizone, and titrated with a solution of mercuric acetate or cadmium sulfate. At the end point there is a sharp color change from yellow to red, owing to the formation of dithizonates.

At a meeting of the Midlands Section on Oct. 12, a paper on "The Analyst's Dilemma: Color or Stability" by R. J. P. Williams, Merton College, Oxford, was presented.

Intense color as the property of a chemical compound, such as a complex ion, is frequently accompanied by instability of the compound. Inspection of the reasons for instability and of the sources of color shows that the two properties are often related. The analyst can use only unstable colored compounds in spot tests. Quantitative reagents demand a stabilization of color. The differences between stable and unstable colored compounds can be explained and the knowledge used to design reagents. Most of the examples discussed were complex ions.

Adaptation of Beckman DU Spectrophotometer to Direct Recording

Lee Cahn, Beckman Instruments, Inc., Fullerton, Calif.

A SIMPLE circuit has been developed for the Beckman DU spectrophotometer which converts the null-balance amplifier to a feedback amplifier capable of operating a conventional millivolt recorder or microammeter. The recorder records "energy" or phototube voltage, as is common with the familiar single-beam type of infrared spectrophotometer. When used with a wavelength drive, continuous spectrograms are obtained. The record thus includes the spectral response of source, detector, and optics.

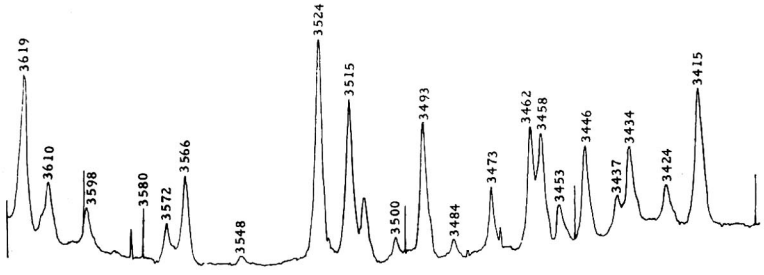

Figure 1. Emission spectrum of iron recorded from DU with adapter

When used at fixed wave length, records may be made of absorption or emission *vs.* time, either for time-varying reactions, or merely to permit visual averaging of detector noise.

Recording is particularly helpful with flame spectrophotometry, providing qualitative information of the sort which recording previously made available in absorption studies. Examples of an iron spectrum (Figure 1) and of a mixture of manganese chloride and potassium chloride (Figure 2) are shown. Averaging of noise is also especially useful in flame work, to extend the limits of detectability. In flame work the single-beam feature is generally found satisfactory.

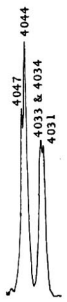

Figure 2. High resolution obtainable with photomultiplier attachment

Resolves 403-mμ Mn line and 404-mμ K line completely; resolves doublets separated by 0.3 mμ

Absorption studies over wide spectral ranges will usually require resetting the slits several times. Narrow ranges can often be scanned without resetting slits. In the ultraviolet, the background changes only threefold between 270 and 220 mμ (Figure 3). While single-beam operation is less convenient, it is often preferable to no recording at all.

It is possible to return to ordinary null-balance operation on the spectrophotometer by means of a switch, for the most careful and precise work.

The amplifier is still of the direct current type, so a certain amount of drift is present. It should not exceed 0.1 to 0.2% T at 100% in 1 minute, if instrument and batteries are in good condition.

In the null position of the null-record switch operation is conventional (Figure 4). The second tube acts as a current amplifier, and the indicating meter is nulled by operating the dark current, sensitivity of slit, and transmittance knobs in sequence. In the record position, the second tube acts as a cathode follower, and its output voltage is fed back to the first-stage grid, to linearize the output, in place of the transmittance voltage. This feedback circuit is similar to the Beckman EASE computer operational amplifier, which is highly linear and stable. The cathode voltage is applied to any potentiometer recorder, with recommended full-scale range 50 mv. A separate sensitivity control, $R21$ is useful for the record position. The DU dark current control is still effective. It is sometimes necessary to readjust it in switching between null and record.

Any potentiometer recorder may be used. If a 0- to 10- or 0- to 50-mv. recorder is used, slit widths narrower than those normally available are obtained at the most sensitive position of the 100% adjustment control. The least sensitive position on 0- to 50-mv. span corresponds approximately to the least sensitive DU position.

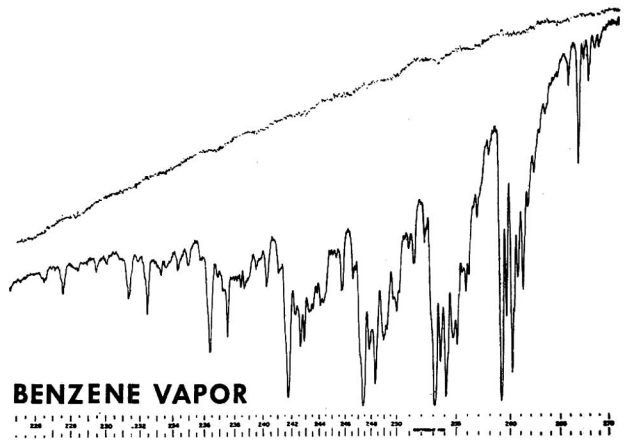

Figure 3. Benzene curve

a. Benzene vapor absorption, 270 to 225 mμ, H_2 lamp and photomultiplier, scanning time 10 minutes
b. Air; background varies only threefold from 270 to 220 mμ
Note adhesive wave-length scale attached to strip chart

An indicating microammeter with a 0- to 100-μa. full scale may be substituted for $R22$, converting the spectrophotometer to a direct reading meter-type instrument. This may be convenient for lower accuracy work, where speed and operator fatigue are important. It is difficult to obtain meters linear to less than ±1%, or about ten times worse than null balance operation.

The circuit may be used with a photomultiplier and/or battery power regulator.

The wave-length drive originally designed for the DK recording spectrophotometer (Bull. 352, Beckman Instruments, Inc., Fullerton, Calif.) adapts readily to the DU instrument. It has five speeds, permitting scanning times of 1, 3, 10, 30, and 100 minutes for 288° of wave-length scale rotation. Because of the nonlinearity of the wave-length scale, the millimicron range covered in 288° varies with the region covered. The shorter times are suitable for liquid samples in the ultraviolet, the longer

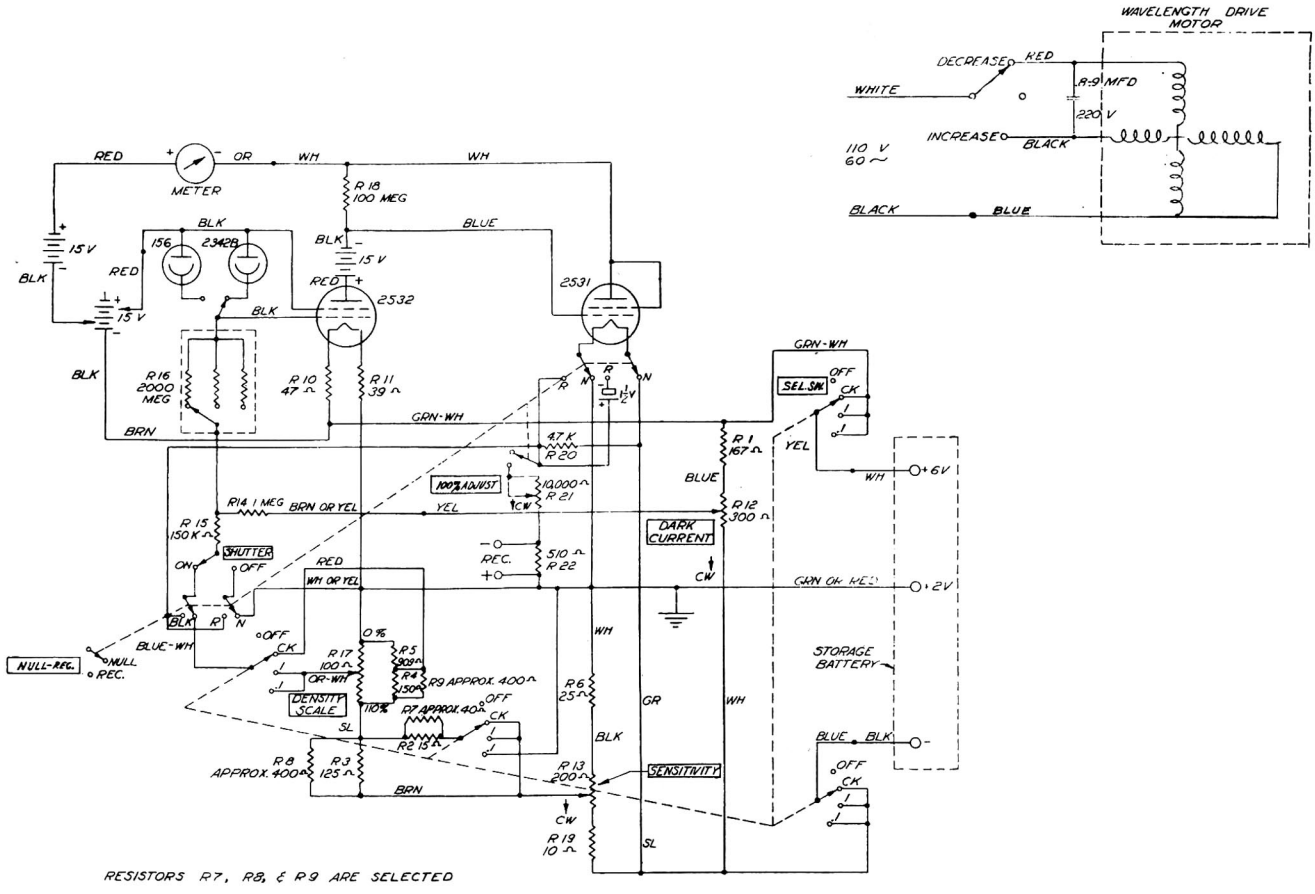

Figure 4. Schematic diagram of modified circuit

for flame spectra at high resolution. If the wave-length drive is used with a recorder having speeds of 2, 4, and 6 inches per minute, the gummed tapes of the DK instrument may be used for an approximate wave-length scale on the strip chart. By the use of special chart drive gears (Part 4892, Beckman Instruments, Inc., Fullerton, Calif.), a wide variety of scanning speeds may be accommodated to the tapes.

Although such a device lacks many of the valuable features found in the double-beam automatic instruments, it appears to be much less expensive, and may find a place in many laboratories where a double-beam instrument would not be justified.

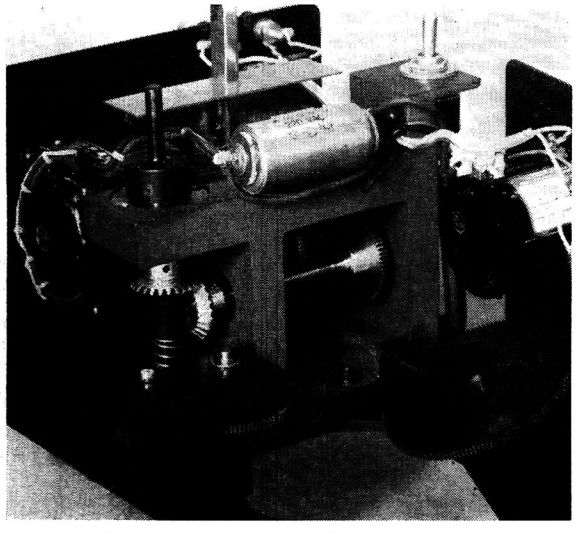

Figure 5. Wave-length drive for DU

Five speeds are available, 5 to 500 minutes full scale. Loose gear on right fits on wave-length knob shaft, is thumbed for manual control

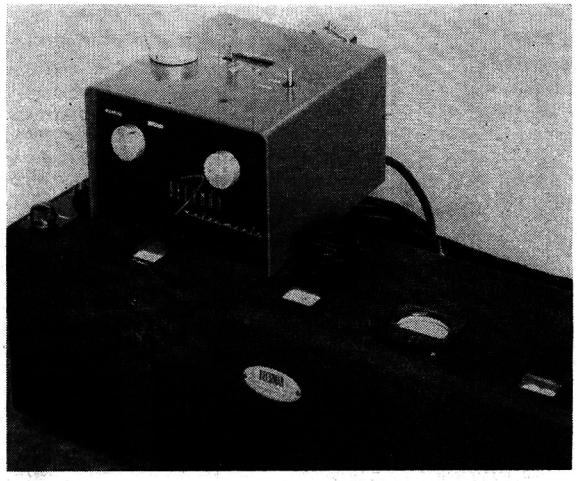

Figure 6. Spectral energy recording adapter installed on DU spectrophotometer

Color Comparator for Determination of Water in Cellophane

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THE speed with which cellophane reaches equilibrium with atmospheric humidity makes necessary an accurate and rapid method for determining water in production control and research. The Karl Fischer titration method, which has been in use in cellophane laboratories for some time, was chosen over distillation methods because of the economy in time and sample size. Various methods for determining the end point of the Karl Fischer titration have been presented. Frediani (*3*) has devised a way to accomplish this amperometrically by the "dead stop" technique. Almy, Griffin, and Wilcox (*1*) have shown that the end point of the titration can be determined potentiometrically as well as colorimetrically. Fischer (*2*) determined the end point using methylene blue indicator, and Whittum (*4*) used gold fluorescent lamps to match the yellow color of the solution of Karl Fischer reagent and water, thus giving a solution of colorless appearance until the end point was reached. The work reported in the present paper was undertaken to find a simple, rapid, and inexpensive means of determining this end point with an accuracy equal to that of one of the above-mentioned techniques.

APPARATUS

The color comparator consists of a box containing a light source from which two light beams emanate. One beam passes through the sample jar, containing solvent and indicator, and the other passes through a 670-mμ filter. The operator simultaneously views both light beams on a ground-glass plate and matches sample color against the standard color while titrating with Karl Fischer reagent. The sample vessel is a 16-ounce screw-cap bottle which rests on a magnetic stirrer. The bottle is fitted with a rubber stopper which contains three holes, one for the buret tip, a second for the Drierite tube, and the third and largest for inserting the test sample. The largest hole is in turn fitted with a rubber stopper which can be temporarily removed for placing the sample in the titration vessel. Subsequent samples can be placed in the titration vessel without removing previous samples or solvent until the vessel is filled or the magnetic stirrer bar becomes clogged. The apparatus is shown schematically in Figure 1.

REAGENTS

Karl Fischer reagent is prepared by dissolving 84.7 grams (0.33 mole) of iodine in a mixture of 269 ml. (3.3 moles) of pyridine and 667 ml. of methanol. The solution is cooled in ice and 64 grams (1 mole) of gaseous sulfur dioxide are added slowly to prevent excessive warming. The reagent is allowed to stand at least 1 day prior to use. Other methods for the preparation of the Karl Fischer reagent may be used.

Methylene blue indicator, 0.1% in pyridine.

PROCEDURE

Methanol is added to the sample vessel until it is half full. Two drops of the methylene blue indicator solution are then added, the vessel is closed with the large stopper, and the magnetic stirrer is set to give a constant rate of agitation. Approximately 0.5-ml. increments of Karl Fischer reagent are added until the color of the sample solution matches the amber color of the standard. The instrument is now ready for a sample determination.

Because of its instability, the Karl Fischer reagent was standardized every day. The solution in the sample vessel was titrated to the end point and a 120- to 150-mg. sample of water was added. The water sample was then titrated and the water titer calculated.

Cellophane samples were analyzed by weighing a film sample containing 20 to 30 mg. of water, stirring the sample for several minutes to extract the water, and then titrating to the end point.

DISCUSSION

The color comparator method is more rapid than an electronic method. Materials other than cellophane have been analyzed readily, including benzene, polyethylene, and paper. Results on these materials agree closely with those obtained using the distillation method. It appears possible to use the method on many other materials. One limitation is the tendency of certain substances, salts such as barium chloride and sodium tartrate, to cloud the solvent, thus masking the end point. With colored solutions the same trouble would be encountered.

Table I. Titration of Water with Karl Fischer Reagent

Sample Weight of Water, Gram	Vol. of Karl Fischer Reagent, Ml.	Water Titer, Mg./Ml.	Deviation from Mean
0.1356	30.10	4.50	−0.06
0.1997	44.28	4.51	−0.05
0.1512	33.90	4.46	−0.10
0.1913	42.30	4.52	−0.04
0.1501	32.40	4.63	+0.07
0.0961	20.40	4.71	+0.15
		Mean 4.56	±0.08

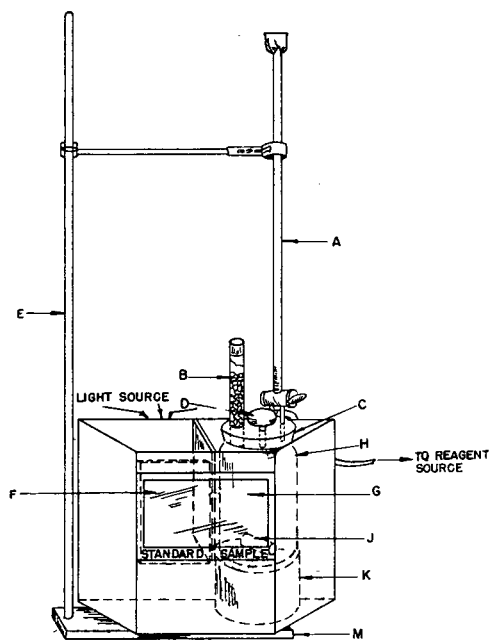

Figure 1. Schematic drawing of color comparator

A. Buret
B. Drierite tube
C. Rubber stopper
D. Sample entrance
E. Ringstand bar
F. Standard viewing screen
G. Sample-viewing screen
H. Sample bottle
J. Magnet for magnetic stirrer
K. Magnetic stirrer
M. Ringstand base

Because any water analysis employing the Karl Fischer reagent requires daily standardization of the reagent, weighed samples of distilled water were analyzed using the color comparator. This test also illustrates the reproducibility of measurement using the color comparator. The weight of water was determined by difference; usually 2 to 4 drops of water were used to keep the weight between 0.1 and 0.2 gram. The data in Table I show a maximum deviation from the mean of 0.15 mg. per ml. and an average deviation from the mean of 0.08 mg. per ml. This amounts to a variation of approximately ±1.7% about the mean, which was considered sufficiently accurate for the analyses for which the color comparator was intended.

A further test of the reproducibility of measurement using this instrument was demonstrated by analyzing duplicate specimens of a number of cellophane samples (Table II). The maximum

Table II. Water Content of Cellophane

Sample No.	Water, %	Mean, %	Sample No.	Water, %	Mean, %
1	2.70 2.76	2.73	7	6.04 5.94	5.99
2	2.51 2.71	2.61	8	7.35 7.47	7.41
3	2.68 2.53	2.60	9	5.90 6.02	5.96
4	2.53 2.45	2.49	10	6.83 6.90	6.86
5	7.17 7.12	7.14	11	6.29 6.35	6.32
6	7.11 6.97	7.04			

Table III. Comparison of Data

	Color Comparator			Electronic Titrator		
Sample No.	Water, % (av. of 4 values)	Av. deviation	% av. deviation	Water, % (av. of 4 values)	Av. deviation	% av. deviation
1	3.9	±0.17	±4.0	4.0	±0.11	±4.4
2	6.1	0	0	5.7	±0.04	±2.8
3	4.8	±0.03	±1.4	5.1	±0.08	±4.1
4	5.5	0	0	5.1	±0.17	±8.6
	Av.	±0.05	±1.3		±0.10	±5.0

deviation from the mean was 0.1% and in the majority of cases measurements within 0.06% of the mean were easily obtainable.

In order to compare the accuracy of measurement possible with the color comparator with that obtainable on a commercially available electronic titrator, specimens of the same cellophane were analyzed on each of these types of instrument. Four separate samples were tested, and four observations were made on each sample using both instruments.

It is readily seen from Table III that the results when using the comparator were equivalent to those obtained using the electronic titrator. The maximum deviation from the mean for the color comparator of ±0.17% water and the average deviation from the mean of ±0.05% water or about ±1.3% compare favorably with a maximum deviation from the mean of ±0.10% water or ±5.0% for the electronic titrator. Samples 2 and 4 for the color comparator data show zero deviation. This is not usual, but may occur with a small number of samples. In a majority of analyses, a reproducibility of ±2% can be expected.

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LITERATURE CITED

(1) Almy, E. G., Griffin, W. C., and Wilcox, C. S., IND. ENG. CHEM., ANAL. ED. 12, 392 (1940).
(2) Fischer, E., *Angew. Chem.* 64, 592 (1952).
(3) Frediani, H. A., ANAL. CHEM. 24, 1126–8 (1952).
(4) Whittum, J. B., *Ibid.*, 23, 209 (1951).

PRESENTED before the Pittsburgh Conference on Analytical Chemistry and Applied Spectroscopy, Pittsburgh, Pa., 1955.

Van Slyke Manometric Apparatus Modified for Determination of Free Amino Nitrogen in Solid Samples

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THE apparatus described by Van Slyke and Neill (*4*) and its use for the determination of amino nitrogen (*3*) have been of great value to the authors. However, in the course of the research at this laboratory it became necessary to determine the free amino nitrogen in wool (*2*), potato granules (*1*), and other solid materials such as poultry meat and feathers. Wool and other fibrous materials could not be introduced into the reaction chamber of the Van Slyke–Neill apparatus. There was great difficulty and loss of many samples in the introduction of potato granules and similar materials into the apparatus.

There was need for a larger opening into the reaction chamber without alteration of the smoothly operating stopcock-reservoir arrangement of the conventional apparatus. Therefore, the reaction chamber was modified by the use of a S 35/25 ball joint as shown in the diagram. The chamber was constructed to have the same length, volume, stopcock-reservoir arrangement, and calibration as the conventional apparatus available commercially. The chamber can be mounted with split rubber stoppers in the conventional apparatus by omitting the jacket.

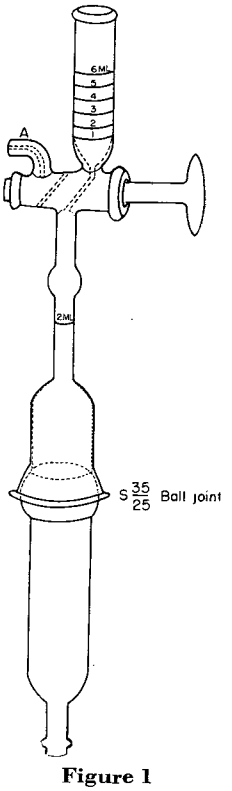

Figure 1

Wool and other solid materials could be easily introduced into this modified reaction chamber. Of almost equal importance was the ease with which the apparatus could be cleaned during the analysis of the sample.

OPERATION

The mercury is lowered below the ball joint and the upper section of the chamber is removed. The sample, 5 ml. of distilled water, and 1 ml. of glacial acetic acid are introduced into the lower section of the chamber. The ball joint is carefully lubricated, the chamber is assembled to form a vacuum seal, and the ball joint is secured with a clamp (not shown in Figure 1).

The standard reaction of the sample with nitrite and the transfer of the reaction gases to the Hempel pipet are carried out as described by Van Slyke (*3*).

While the nitrogen is in the Hempel pipet the mercury is lowered below the ball joint, the reaction chamber is opened, and the sample residue is removed from the lower section of the chamber by suction. Water is added, and scrubbing with a rubber policeman is applied as needed. The upper section of the chamber can be cleaned in any suitable manner. After cleaning, the ball joint is lubricated and the reaction chamber is assembled as before. The analysis is completed as described by Van Slyke (*3*).

The discharge tube, *A*, is included in the modified apparatus, so that it can be used in the manner described by Van Slyke (*3*) for the analysis of samples that do not require opening of the ball joint.

LITERATURE CITED

(1) Hendel, C. E., Burr, H. K., and Boggs, M. M., U. S. Dept. Agr., Western Regional Research Laboratory, Albany, Calif., AIC-303, 1951.
(2) Jones, W. H., and Lundgren, H. P., *Textile Research J.* 21, 20–9 (1951).
(3) Van Slyke, D. D., Jr., *J. Biol. Chem.* 83, 425–47 (1929).
(4) Van Slyke, D. D., and Neill, J. M., *Ibid.*, 61, 523–73 (1924).